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Recent Trends and Applications of Leguminous Microgreens as Functional Foods

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Preface

Microgreens are the name given to all those small, soft, green parts that develop from seedlings and do not bear any real leaves. These microgreens are known to have high nutritional content and composed of phytoconstituents such as carotenes, phenolics, polysterols, etc. which impart them for their usage in dietary programs. Leguminous microgreens provide high nutritional value by enhancing dietary fiber, vitamins, and minerals, and are one of the most important sources of plant protein in the human diet. These leguminous microgreens play a significant role in fulfilling the nutritional requirement of World's population and will assist in completing the United Nations' global goal of "zero hunger" (Sustainable Development Goal 2). Therefore, the present book has been designed to increase the awareness of leguminous greens as well as their nutritional and dietary among the scientific community. An important part of the book delineates the innovative cultivation strategies for increasing the production of biofortified leguminous microgreens. The book showcases application of biological methods such as microbial consortia, Plant Growth Promoting Rhizobacteria (PGPR), Arbuscular Mycorrhizal Fungi (AMF), etc. in agricultural fields to improve the crop production as well as nutrient quality of leguminous crops. Concomitantly, the book comprehensively discusses about various conventional, molecular, and biotechnological approaches such as breeding, genetic engineering, Clustered regularly interspaced palindromic repeats-CRISPR associated (CRISPR-Cas), etc. that have been adopted during recent years for enhanced cultivation of leguminous crops. The book also highlights on the emerging application of nanotechnology, hydroponics, and aquaponics systems for the development of nutritionally rich leguminous crops. The

present book will prove to be valuable tool for anyone looking to gain a comprehensive understanding of this growing sector of leguminous microgreens and their application as a biofortified food.

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Nutritional Profile of Leguminous Microgreens: Seed to Superfood

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Abbreviations

AsA Ascorbic acid

DPPH 2-diphenyl-1-picryl-hydrazy

ILDIS International Legume Database & Information Service

NCDs Non-communicable diseases

TAA Total antioxidant activity

TAC Total ascorbic acid

TFC Total flavonoids content

TPC Total phenolics content

WHO World Health Organization

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1 Introduction

Today our society believes in ‘Eat to live, not live to eat’. With the global urban population expansion, there is a huge ever increasing demand for a more ready to hand, sustainable, and nutritious food supply. It is estimated that about three billion people cannot afford a healthy diet around the world which accounts to mostly people living in sub-Saharan Africa and South Asia (FAO 2021; Herforth et al. 2020). Non-communicable diseases (NCDs) such as diabetes, cardiovascular disease, hypertension, stroke, cancer including obesity are multiplying globally mainly due to imbalances in diet. Growing public health concerns have prompted the search for novel food sources. The World Health Organization (WHO) recommended daily intake of 400 g of edible fruits and vegetables to check NCDs and diminish several micronutrient deficiencies (WHO 2021). While there’s widespread recognition of the importance of fruits and vegetables for a balanced diet, there are challenges in ensuring that current and projected fruit and vegetable production would fail to meet healthy consumption levels (Siegel et al. 2014; Bahadur et al. 2018).

In the modern-era of exploration and scientific advancements, globally, search for a more novel source of healthy, functional and available food, specially, post-COVID-19 pandemic has made the society show tremendous interest in

eating fresh and healthy foods, such as sprouted seeds and micro-greens (Ebert 2022). The use of micro greens is gaining popularity due to their concentrated nutrient content and versatility in culinary applications and therapeutic benefits. Compared to sprouts, which are germinated seeds harvested just after the root emerges; microgreens are more mature and have a more developed structure, including stems and leaves. On the other hand, they are smaller than baby vegetables or greens, having a central stem with two fully developed, non-senescent cotyledon leaves and one pair of small true leaves (Di Gioia and Santamaria 2015; Lenzi et al. 2019; Treadwell et al. 2020; Turner et al. 2020). We consume mainly the stem, cotyledons and first true leaves.

Microgreen's compact size, rapid growth, and minimal requirements make them ideal candidates for cultivation in controlled indoor environments therefore considered as part of international movement towards "controlled environmental agriculture" (CEA) towards achieving global food security (Riggio et al. 2019). Carefully selecting the growing media, high-quality seeds, and implementing biofortification techniques, growers can enhance the nutritional profile of microgreens even further (Sharma et al. 2022). The leguminaceae, being one of the largest families of flowering plants having about 18,000 species classified into 650 genera, and its global distribution highlights the adaptability and ecological importance of leguminous plants, which play crucial roles in ecosystem dynamics, soil fertility, and human food security across the planet (except Antarctica) (ILDIS 2017). Among all the microgreens grown, leguminous microgreens has gained special attention due to their high protein, carbohydrate, dietary fiber (Bubelova et al. 2018), abundance of minerals and secondary metabolites and therefore considered valuable components of staple and functional foods. In addition, they are rich in secondary metabolites (antioxidants, flavonoids, carotenoids and chlorophyll) which cannot be synthesized in the body. These compounds have the potential to counteract several nutritional deficiencies (Klopsch et al. 2018).

Leguminous microgreens, like other microgreens, are an emerging superfood, gaining attention for their nutritional density and culinary versatility. Leguminous microgreens, such as alfalfa, clover, lentil, chickpea, pea, mung bean, blackgram, cowpea, pigeon pea, fenugreek, horsegram etc., are particularly rich in protein, fiber, and essential amino acids, making them an excellent addition to plant-based diets. The emergence of leguminous microgreens in the food and nutritional security could indeed revolutionize how we approach vegetable consumption. Their rapid growth cycle, high nutritional value, and ability to be grown year-round in small spaces make them an attractive option for both home cooks and commercial food producers. As more people become aware of the health benefits and culinary potential of leguminous microgreens, they're likely to become a staple in kitchens around the world, potentially reshaping our diets and promoting more sustainable and nutrient-rich food choices.

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2 Why Microgreens

Recently, a large cognizance of masses has started giving more importance to the well-being of their health, especially due to the outbreak of various kinds of unexpected disorders and their constant health deterioration (Luo and Wang 2021). Rapid shrinkage of the land resources, healthy diet habits, lifestyle changes, etc. are also some of the factors which progressively resulted in increased interest of the people to include fresh fruits and vegetables and other kinds of green leafy vegetables as part of their daily diet to ensure adequate nutrition. Microgreens have emerged as the potential therapeutic functional foods to improve the overall health by dietary supplementation (Sharma et al. 2022). Growing microgreens have an advantage as their growth period is comparatively shorter with a lower maintenance compared to matured green plants and their produce, such as vegetables and fruits (Sharma et al. 2020).

As microgreens are considered a functional food, or say "Super food" can be easily produced in the urban and peri-urban

areas with a minimum or without any external nutrients in a limited space with a short growth cycle (Wojdyło et al. 2020). On the other hand, for the production of mature plants we require a growing medium, proper light, external inputs such as fertilizers, pesticides, irrigation etc., at regular interval from sowing till harvest with other intercultural operations and an expanded time period to grow (Di Bella et al. 2020). The different characteristics of microgreens and mature plants are discussed in Table 1.

Table 1 Characteristics of microgreens and mature plants

Conditions	Microgreens	Mature plants
Height	3–10 cm	Several cm
Production time	7–21 days	Several months
Cultivation system	Can be grown in soil or entirely in medium.	Require medium to grow or grown mostly in soil fields.
Light requirements	Yes, requires light source.	Yes, requires light source.
Root appearance	Small roots with root hairs.	Mature root system
Agrochemicals use	Can be grown chemical free.	Can be grown organically or using chemicals.
Moisture/water use	Very small amount of water is required.	Huge amount of water is required.
Land space	Very small space is required for large scale production also	Grown over acres of free and open-spaced lands
Plant growth level at harvest time	Full development of cotyledons with one or two true leaves	Full development of mature plant that may bear fruits or vegetables.
Harvest type	Harvesting is done by removing the roots.	Harvesting is done by cutting the roots either manually or mechanically

Source: Bhaswant et al. (2023)

3 Nutritional Value of Leguminous Microgreens

Following the COVID-19 pandemic, there was a global surge in awareness of the need for a balanced diet and the introduction

of novel, health-promoting foods including sprouted seeds and microgreens (Ebert 2022). Due to their apparent availability of several nutritional components, including high levels of protein, carbohydrates, and dietary fibre, legumes are regarded as a high-energy food source on a global scale (Bubelova et al. 2018). Because of their high nutritional content, legumes such as beans, lentils, chickpeas, fenugreek etc. can also be used to produce microgreens. Secondary metabolites, including antioxidants, flavonoids, carotenoids, and chlorophyll concentration, are abundant in legume microgreens. Furthermore, compared to their mature counterparts, microgreens might have higher concentrations of vitamins, minerals, and phytochemicals (Yadav et al. 2019a, b; Xiao et al. 2012). According to Klopsch et al. (2018), these nutrient-dense microgreens are thought to be the “superfoods” of the future since they enhance palatability and address nutritional inadequacies. While a plethora of research has been published on the bioactive and antioxidant characteristics of cereals (Niroula et al. 2019), relatively little is known about leguminous microgreens (Table 2).

Table 2 Composition of phytochemicals in different microgreens

Sl. No.	Name of plant	Nutritional value	Source
1	Soybean	1. Phenolics—5.5 mg/g	Zhang et al. (2020)
		2. Flavonoids—68 mg/g	
2	Chickpea	1. TAA—394.6 mg TE/100 g	
		2. TPC—32.20 mg GA/100 g	Sanyukta et al. (2023)
		3. TFC—7.57 mg QE/100 g	
		4. TAC—13.0 mg/100 g	Altuner et al. (2022)
		5. Total carotenoids—4.523 µg/g	
		6. DPPH—90.60%	

Sl. No.	Name of plant	Nutritional value	Source
3	Green peas	1. Sugars—0.5 mg/g	
		2. Ascorbic acid—9.1 mg/g	
		3. Polyphenol—108.5 mg/100 g	
		4. Chlorophylls—522.75 µg/g	Wojdyło et al. (2020)
		5. Carotenoids—2794.4 µg/g	
		6. Amino acids—397.9 mg/100 g	
4	Clover	1. TAA—4789.4 mg TE g-1 DM	Altuner et al. (2022)
		2. TPC—791.8 mg GA 100 g-1 DM	
		3. TFC—472.9 mg QE 100 g-1 DM	
		4. TAC—11.6 mg 100 g-1 DM	
		5. Total carotenoids—4.365 µg/g	
5	Alfalfa	1. TAA—990.4 mg TE g-1 DM	
		2. TPC—120.16 mg GA 100 g-1 DM	Fabek Uher et al. (2023)
		3. TFC-60.11 mg QE 100 g-1 DM	Altuner et al. (2022)
		4. TAC—9.8 mg 100 g-1 DM	
		5. Total carotenoids—5.232 µg/g	
		6. AsA—56.75 mg/100 gfw	
6	Mung bean	1. TAA—554.9 mg TE g-1 DM	
		2. TPC—402.8 mg GA 100 g-1 DM	
		3. TFC—111.7 mg QE 100 g-1 DM	Altuner et al. (2022)
		4. TAC—13.6 mg 100 g-1 DM	
		5. Total carotenoids—1.769 µg/g	
7	Lentils	1. TAA—742.9 mg TE g-1 DM	
		2. TPC—497.3 mg GA 100 g-1 DM	
		3. TFC—418.7 mg QE 100 g-1 DM	Altuner et al. (2022)
		4. TAC—12.5 mg 100 g-1 DM	
		5. Total carotenoids—7.015 µg/g	
8	Cowpea	1. TAA—410.8 mg TE g-1 DM	
		2. TPC—219.5 mg GA 100 g-1 DM	
		3. TFC—113.1 mg QE 100 g-1 DM	Altuner et al. (2022)
		4. TAC—12.4 mg 100 g-1 DM	
		5. Total carotenoids—4.041 µg/g	

Sl. No.	Name of plant	Nutritional value	Source
9	Fenugreek	1. Protein—2.33 mg/g	Nautiyal et al. (2022)
		2. Carbohydrates—3.43 mg/g	
		3. Ascorbic acid—12.14 mg/g	
		4. TPC—63.91 mg GA 100 g-1 DM	Ghevariya et al. (2023)
		5. TFC—19.07 mg QE 100 g-1 DM	
		6. Total tannins—0.83 mg/g	

4 Potential Health Benefits of Leguminous Microgreens

As a relatively recent culinary and nutritional phenomenon, microgreens also known as “living superfoods”—have gained attention as a possible solution for addressing the dietary demands of urban dwellers. Studies have shown the anti-inflammatory, anti-cancer, anti-bacterial, and anti-hyperglycemic properties of microgreens (Zhang et al. 2021) which may be useful in the treatment and prevention of chronic diseases like as obesity, type 2 diabetes, and cardiovascular disorders (Wadhawan et al. 2018). In this context, the tiny-seeded legumes offer unmarked sources of possible components for adding nutrients, minerals, and bioactive compounds to traditional or staple cuisines (Butkute et al. 2019). Regarding possible health benefits, such as lowered risk of diabetes, cancer, heart disease, oxidative stress, low-density lipoprotein (LDL) cholesterol, and other conditions, legumes are significant (Hu 2003; Frede et al. 2017). These findings add to the appeal of microgreens as a novel functional food that is good for human health. For instance, peas and their products have been shown to provide a number of health advantages, including the ability to regulate the effects of metabolic syndrome and be antioxidant, anti-inflammatory, antibacterial, and anti-renal fibrosis (Wu et al. 2023). The amount of total phenolics, vitamins, and antioxidant activity rose in the order of raw seeds, sprouts, and microgreens in the Fabaceae vegetables chickpea and mungbean (Kurian and Megha 2020). When compared to

ungerminated seeds, the sprouting of mungbean seeds enhanced the amounts of total phenolic and flavonoid (TF) and antioxidant activity (AA) (Pajak et al. 2014). Furthermore, reports revealed (Wojdyło et al. 2020) the sprouts of several legume microgreens showed the highest inhibition and α-glucosidase, suggesting an alternate strategy for avoiding diabetes mellitus of α-amylase (Fig. 1 and Table 3).

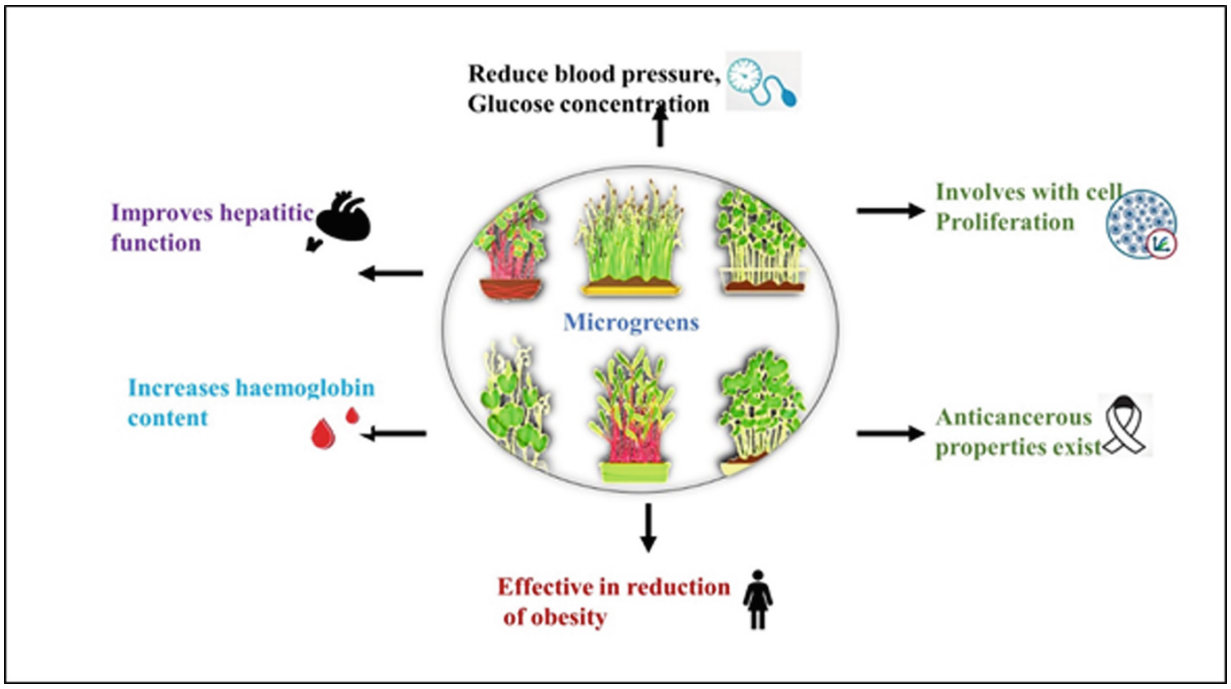


Fig. 1 Potential health benefits of microgreens

Table 3 Health benefits of leguminous microgreens

Sl. No.	Legume microgreen	Health benefits of legume microgreens	References
1	Soyabean	Blue light or UV light helps to increase antioxidant activity in respect to level of phenolic compound	Zhang et al. (2019)
		Valuable sources of Protein (14%), Zn (45%) and Ca (72%)	Ebert et al. (2017)

Sl. No.	Legume microgreen	Health benefits of legume microgreens	References
2	Fenugreek	Higher level of glucose content and enzymatic activity can maintains the blood sugar, that helps diabetic patient	Wadhawan et al. (2018)
		Contain fiber that significantly reduce the glycemic index, that helps to check blood sugar level	Sowmya and Rajyalakshmi (1999)
		Fiber content helps to moderate metabolism of glucose in digestive tracts; therefore, it reduces the glucose absorption in the intestine	Olaiya and Soetan (2014)
		Lower down the serum cholesterol, triglyceride and low-density lipoprotein in hypercholesterolemia suffering patient	Mathern et al. (2009)
		Antioxidant activity with rich content of protein, vitamin and minerals	Altuntaş et al. (2005) and Nautiyal et al. (2022)
		Sapogenin found that is useful for preparation of anti-cancerous compound	Aggarwal and Shishodia (2006)
3	Pea	Rich in natural antioxidants, including phenolic compounds carotenoids, chlorophylls, ascorbic acid etc.	Wojdyło et al. (2020)
		Antiobesity activity, antidiabetic activity, anticholinergic activity	
		Anti-inflammation, anti-cancer, anti-osteoporosis	Wu et al. (2023)
4	Mung bean	Antiviral activity	Hafidh et al. (2015)
		Vitamin, total phenol and total flavonoid	Pajak et al. (2014)
5	Alfalfa	Inhibitory effect against cholesterol absorption in blood plasma	Harwood (1993)
		Anticancer and antimicrobial activity	Cui et al. (2020)
		Antinflammetory, antidiabetic, antioxidant activity	da Silva et al. (2013)
		Activities increase enzymatic function	El-Deeb et al. (2016)
		Antiobesity activity	Hong et al. (2011)
6	Chick pea	Rich in vitamin content and antioxidant activity	Kurian and Megha (2020)
7	Red clover	Total phenolic compounds, antioxidant and antidiabetic activities	Altuner et al. (2022)

Sl. No.	Legume microgreen	Health benefits of legume microgreens	References
8	Rajmah	Total antioxidant activity	Sreeramulu et al. (2009)

5 Consumer Acceptance of Leguminous Microgreens

Understanding the factors influencing the acceptability of leguminous microgreens involves considering various aspects such as taste, texture, appearance, nutritional content, and culinary versatility. There are some common leguminous microgreens species like pea, lentil, mung bean along with their characteristics that influence acceptability. Consumer acceptance of leguminous microgreens, such as those from the Fabaceae family, can vary based on several factors including flavour, texture, appearance, and nutritional value. Here are some considerations regarding consumer acceptance of leguminous microgreens.

5.1 Flavour

Leguminous microgreens may possess unique flavour profiles ranging from nutty to earthy. Consumer acceptance depends on whether these flavours are perceived as pleasant and complementary to various dishes.

5.2 Texture

The texture of leguminous microgreens can vary from tender to slightly crunchy, depending on the species and maturity stage. Consumers may prefer microgreens with a desirable texture that adds interest to salads, sandwiches, and other culinary creations.

5.3 Appearance

Visual appeal plays a significant role in consumer acceptance. Leguminous microgreens often exhibit vibrant green colors with delicate leaves, which can enhance the visual appeal of dishes and contribute to a fresh and appetizing presentation.

5.4 Nutritional Value

Consumers increasingly seek nutritious foods, and leguminous microgreens are valued for their high nutritional content, including protein, fiber, vitamins, and minerals. Highlighting the nutritional benefits of leguminous microgreens may positively influence consumer acceptance.

5.5 Culinary Versatility

Microgreens that can be easily incorporated into a variety of dishes are likely to be more accepted by consumers. Versatility allows for creativity in cooking and meal preparation (Turner et al. 2020).

Highlighting the nutritional benefits of leguminous microgreens may positively influence consumer acceptance. By considering these factors and experimenting with different leguminous microgreens species, researchers can better understand consumer preferences and tailor production methods to meet market demands. Additionally, conducting sensory evaluations and consumer surveys can provide valuable insights into acceptability and preferences (Tan et al. 2020).

However, it's essential for consumers to broaden their perception of palatability to include vegetables with less agreeable or more intense flavour profiles, such as those with bitterness or sourness. These flavour profiles are often associated with high concentrations of chemopreventive phytonutrients, which contribute to the health-promoting properties of these food (Michell et al. 2020).

Despite their potential health benefits, the presence of intense or unfamiliar flavours can act as a barrier to consumer acceptance and may limit the diversification of the genetic basis of food production. Encouraging consumers to appreciate the nutritive attributes of vegetables, including microgreens, regardless of their taste profiles, can help promote a more diverse and nutritious diet.

Educational initiatives, culinary innovations, and marketing strategies that highlight the nutritional benefits and culinary versatility of microgreens and other vegetables can help overcome taste-related barriers and encourage consumers to

embrace a wider range of foods for their health and well-being (Xiao et al. 2015).

Overall, ensuring consumer acceptance of leguminous microgreens involves understanding and catering to consumer preferences regarding flavours, texture, appearance, and nutritional value. By highlighting the appealing attributes of leguminous microgreens and providing guidance on their culinary uses, producers can enhance their marketability and appeal to a broader consumer base (Michell et al. 2020).

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6 Potential Underutilized Legume Crops for Microgreens

Exploiting plants with better nutritional quality, capacity to withstand the fluctuating climatic conditions, better adaptation, resisting biotic and abiotic stresses coupled with large-scale production and nutritional diversity, are the key requirements at present. In order to meet the global food demands, focus should be on discovering potential crops which have been neglected and underexploited but have the inherent capacity to enhance food and nutritional security especially in the developing countries. As we know, Legumes are the prime source of protein for the majority of the population, hence, efforts need to be undertaken to exploit the lesser-known legumes to be grown as microgreens which will diversify our food-basket and address the food and nutritional issues of the community (Popoola et al. 2019).

Common legumes which have gained popularity for growing as microgreens include chick pea (*Cicer arietinum* L.), pea (*Pisum sativum* L.), lentil (*Lens culinaris* L.), mung bean (Vigna mungo L.), cowpea (*Vigna unguiculata* L.), groundnut (*Arachis hypogaea* L.), common beans (*Phaseolus vulgaris* L.) and soybean (*Glycine max* L.) among others. Minor legumes being less known, less exploited and is neglected. Some of the underutilized legume crops include bambara groundnut (*Vigna subterranea* L.), winged bean (*Psophocarpus tetragonolobus* L.), rice bean (*Vigna angularies* L.), lima bean (*Phaseolus lunatus* L.), Kidney bean (*Phaseolusvulgaris*), jack and sword bean

(*Canavalia sp.*), Adzuki beans (*Vigna angularis*), African yam bean (AYB) (*Sphenostylisstenocarpa* Harms), marama bean (*Tylosema esculentum* L.) and lablab (*Lablab purpureus* L.) (Popoola et al. 2017; Ojuederie et al. 2014). These species of underutilized legumes have a huge potential as grain legumes to boost protein security in period of hunger and malnutrition in many under developed and developing countries.

The studies of Konyeme et al. (2020) identified the rich nutritional value of tubers, while the study of Ojuederie et al. (2020) confirmed that humans and livestock can safely consume these underutilized legumes in their day to day life. Growing demand for food legumes as one of the essential nutrient and conventional food with many health and pharmacological relevance is ever-increasing (Tadele and Bartels 2019). Many researchers have widely reported and discussed the diverse nutritional contents of the seeds that varies in different accessions of the same and different species (Bourque et al. 2016; Huang et al. 2018; Omotayo and Aremu 2021; Popoola et al. 2022). Further this can be exploited by scientists and breeders to enhance yield, taste and value chain for the food and confectionary industries. The renewed efforts in this direction will be to evolve strong research and development between industries and the academia to exploit these grain legumes to be used for growing microgreens and find the nutritional profile of these selected potential underutilized plants both for its palatability and sensory evaluation. Table 4 summarizes the nutritional profile of these selected underutilized legume crops with a possibility to be grown as microgreens.

Table 4 Nutritional profiles of the selected underutilized legumes' raw, mature seeds, (per 100 g)

Nutrient contents	**ADB**	**AYB**	**BG**	**JB**	**KB**	**LB**	**MB**	**RB**	**WB**
Protein (g)	20.36	22.46	18.8	20.90	23.00	7.80	34.71	20.50	29.60
Carbohydrate (g)	62.26	53.68	61.30	60.61	70.48	20.90	18.9	51.31	41.70
Moisture (g)	13.07	9.53	2.10	2.19	Nr	Nr	2.80	Nr	Nr
Ash (g)	3.85	4.28	2.40	3.45	5.13	1.15	3.19	Nr	3.98

Nutrient contents	ADB	AYB	BG	JB	KB	LB	MB	RB	WB
Fat (g)	0.45	3.59	6.20	1.59	1.38	0.38	40.06	0.60	16.30
Total dietary fibre	7.30	7.30	5.50	3.98	20.93	7.00	50.81	13.10	25.90
Water (g)	13.40	61.50	10.30	Nr	59.00	69.80	Nr	Nr	8.34
Energy (kcal/100 g)	334.80	333.67	367.00	338.00	386.39	115.00	544.57	318.00	409.0
Folates (B9) µg	0.62	0.10	0.25	0.40	1.11	83.00	0.14	Nr	45
Thiamine (B1) (mg/100 g)	0.46	0.19	0.61	0.51	0.50	0.161	0.38	Nr	1.03
Niacin (B3) (mg/100 g)	2.63	0.07	1.80	1.54	0.51	0.421	0.06	Nr	3.09
Riboflavin (B2) (mg/100 g)	0.22	0.20	0.31	0.20	0.03	0.055	0.06	Na	0.45
Vitamin B6 (mg/100 g)	0.35	0.10	0.44	0.51	4.67	0.161	9.21	Na	0.175
Vitamin A (mg/100 g)	11.39	Nr	Nr	Nr	0.00	0.00	0.27	Na	Nr
Vitamin C (mg/100 g)	0	12.97	0.27	8.10	0.55	0.00	0.00	Na	0
Vitamin D (mg/100)	0.00	0.00	3.42	0.00	0.00	0.00	132.9	Na	Nr
Vitamin E (mg/100 g)	Nr	0.19	Nr	Nr	0.10	0.18	6.27	Na	Na
Vitamin K (mg/100 g)	Nr	Nr	0.001	Nr	14.90	2.00	0.22	Na	Na
Pantothenic acid (B5) (mg/100 g)	1.47	Nr	1.80	Nr	0.40	0.422	Nr	Na	0.795
Sodium (mg/100 g)	5.00	1.00	3.60	2.53	53.48	2.00	63.75	32.00	38
Calcium (mg/100 g)	66.00	15.00	1.60	3.21	104.12	17.00	241.00	340.00	440
Copper (mg/100 g)	1.09	0.29	0.09	0.43	0.40	0.235	1.04	1.12	2.88
Iron (mg/100 g)	4.98	1.50	5.52	0.83	7.00	2.39	3.95	5.80	13.40

Nutrient contents	ADB	AYB	BG	JB	KB	LB	MB	RB	WB
Magnesium (mg/100 g)	127	69.00	7.58	1.95	118.95	43.00	274.50		179
Manganese (mg/100 g)	1.73	3.35	0.26	0.35	0.80	0.516	1.85	0.68	3.72
Phosphorus (mg/100 g)	381	99.00	32.50	1500	251.30	111.00	454.00	Na	451
Potassium (mg/100 g)	1254	419.00	183.00	5.93	1517.36	508.00	895.00	Na	977
Zinc (mg/100 g)	5.04	0.78	0.27	2.90	2.38	0.95	6.20	3.39	4.48
Selenium (mg/100 g)	3.10	150.00	Nr	Nr	2.10	4.50	0.08	Nr	8.20
Beta-carotin (µg)	Nr	7.00	0.47	Nr	Nr	0.00	Nr	Nr	Nr

N.B: Values adopted from the United States Department of Agriculture (USDA), Baiyeri et al. (2018), Charrondière et al. (2020), and Nnamani et al. (2018)
ADB Adzuki beans (*Vigna angularis*), *AYB* African yam bean (*Sphenostylis stenocarpa*), *BG* Bambara groundnut (*Vigna subterranea*), *JB* Jack bean (*Canavalia ensiformis*), *KB* Kidney bean (*Phaseolus vulgaris*), *LB* Lima bean (*Phaseolus lunatus*), *MB* Marama bean (*Tylosema esculentum*), *RB* Rice bean (*Vigna umbellata*), *WB* Winged bean (*Psophocarpus tetragonolobus*), *Nr* Not reported, *Na* Not available

7 Sensory Attributes of Different Microgreens

Microgreens are garnering increasing attention from consumers due to their perceived freshness and health benefits attributed to their dense concentration of secondary metabolites. These young greens not only enhance the nutritional profile of the human diet but also contribute vibrant colours and flavours. Unlike sprouts, microgreens are consumed without their roots and require additional time to develop larger, colourful, and flavourful leaves under optimal light conditions (Brentlinger

2005). Despite their immaturity, microgreens possess intense flavours, striking colours, delicate textures, and a wealth of phytonutrients. These appealing sensory characteristics have earned microgreens a prominent place in the repertoire of chefs for garnishing soups, sandwiches, beverages, and more, and have led to their inclusion in organic farming stores.

Numerous species are utilized for microgreens production, spanning both commercial cultivars and locally sourced varieties. These species belong to various botanical families, including: Leguminacea, Amarillydaceae, Amaranthaceae, Apiaceae, Asteraceae, Brassicaeae, Cucurbitaceae, Fabaceae, Lamiaceae, Oxalidaceae, Poaceae, Polygonaceae, and Portulacaceae (Kyriacou et al. 2017).

Microgreens contain a higher concentration of essential phytonutrients compared to their mature counterparts and dense phytochemical composition has attracted the attention of nutrition researchers which has made microgreens popular among food technologists, urban farmers, and consumers alike.

The sensory attributes of microgreens, including taste and olfactory sensations, are perceptible by the human organoleptic system. This sensory information contributes to our knowledge and appreciation of the unique flavours, textures, and aromas that microgreens offer, further enhancing their appeal in culinary applications and dietary practices (Di Gioia et al. 2017). Sensory attributes play a crucial role in determining the acceptability of a food product, and they are closely intertwined with its chemical composition. For example, the phenolic content of microgreens contributes to attributes such as astringency, sourness, bitterness, and sweetness. Incorporating microgreens into salads is recognized as a smart strategy to enhance the quality and visual appeal of food without compromising its overall taste.

Numerous studies have investigated how sensory attributes and visual appearance influence consumer perception of microgreens, including comparative studies at different maturity stages. However, there is still a need for further research to assess the sensory attributes and acceptance of many leguminous microgreen species (Renna et al. 2017). Among

these attributes, appearance tends to dominate consumer choice, while other sensory attributes sustain future purchases. Therefore, enhancing the sensory appeal of microgreens through factors like colour, texture, and flavour can significantly influence consumer acceptance and promote their consumption as part of a healthy and sustainable diet.

Microgreens present a diverse array of colours, flavour, and textures, ranging from green to yellow, red, crimson, or even multi-coloured. While visual appearance is often appreciated by consumers, studies suggest that overall consumer acceptance is primarily influenced by the flavours and texture of microgreens (Kyriacou et al. 2017; Di Gioia et al. 2017; Renna et al. 2017).

Research indicates that factors such as astringency, sourness, and bitterness significantly impact consumer acceptability. Generally, microgreens with lower levels of these attributes tend to be more favourably received by consumers. While various species of microgreens are continually being tested for their agronomic and nutritional suitability, it's evident that consumer acceptance based on sensory characteristics—particularly appearance, texture, and flavour—is crucial for their success in the marketplace. Therefore, understanding and optimizing these sensory attributes are essential for ensuring the widespread adoption and popularity of microgreens among consumers (Di Gioia et al. 2017).

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8 Nutritional Values and Phytochemicals Present at Various Growth Stages of Leguminous Microgreens

Now days dietitians are including microgreen in daily food habit as a way to promote wellness and health due to having high nutrients and bioactive compounds such as flavonoids, hydroxycinnamic acids, vitamins and glucosinolates, minerals, and carotenoids (Kyriacou et al. 2019). These phytochemicals appear to be essentials in shielding the body from several chronic disease like cancer, cardiovascular disorders and diabetes (Choe et al. 2018; Villano et al. 2019). Furthermore, it is recognised that the sprout and microgreen contained a low

calorific value (29–128 kcal/100 g) and low glycemic index (Choe et al. 2018; Villano et al. 2019).

According to the report of Ebert et al. (2017), sprouting mungbean contained 2.7 fold higher vitamin C content compared to mature mungbean grain. Relatively old line of mungbean grain had higher level of protein, calcium (Ca), iron (Fe), zinc (Zn), carotenoid and vitamin C content compared to fully mature improve lines of mungbean. Additionally vegetable stage of soybean had more nutritional value characterised by protein (14% increase), Zn (45%), Ca (72%), and Fe (151%) compared to soybean sprouts (Ebert et al. 2017).

In contrast to other methods like dehulling, boiling, autoclaving and microwave cooking, germination of seeds has shown to increase the nutritional value of legumes by activating enzymes that decrease or eliminate the activity of phytic acid, stachyose and raffinose in mungbean. On the other hand, germination also helps to better retain many minerals (Mubarak 2005). In terms of nutritional aspects, protein and mineral content in legume microgreens like, lentil, chickpea and fenugreek are highly beneficial and good for health (Table 5).

Table 5 Protein content and mineral composition of dried microgreen of legume crop

Legume microgreen	Protein content	Mineral composition (µg/g)					References
		Fe	Zn	Mg	Ca	K	
Lentil	41.03 ± 0.76 (%)	1.921 ± 0.02	0.502 ± 0.001	28.72 ± 1.29	97 ± 3.4	156 ± 5.5	Ghumman et al. (2017) Santos et al. (2020)
Chikpea	39.293 ± 0.28 (%)	1.762 ± 0.02	0.522 ± 0.001	29.34 ± 1.47	56 ± 3.5	276 ± 1.2	
Fenugreek	5.73 ± 0.03 (µg/g)	82.32 ± 1.22	34.92 ± 052	1645.0 ± 24.92	7658.93 ± 79.58	–	Khoja et al. (2020) and Eswaranpillai et al. (2023)

Report of Taraseviciene et al. (2009) concluded that protein hydrolysis, synthesis, and re-arrangement that involved mobilization of protein reserves in cotyledons and synthesis of new proteins, probably responsible for changes of amino acid content at the time of germination that necessary for the sprout

growth. Furthermore, the amino acids produced by hydrolysis of the protein reserves are not used solely for the synthesis of new components but may also be used as energy sources, especially at early stages of germination. The most abundant free amino acid in green peas (*Pisum satuvum*) microgreens is L-asparagine (Table 6) (Wojdyło et al. 2020).

Table 6 Levels of various free amino acid (mg/100 g fw) composition in microgreens of green peas (*Pisum satuvum*)

Legume microgreen	Amino acid	Content	References
Green peas (*Pisum satuvum*)	L-Histidine	7.7 ± 1.1	Wojdyło et al. (2020)
	L-Asparagine	106.2 ± 4.1	
	L-Arginine	83.2 ± 2.5	
	L-Serine	12.9 ± 1.0	
	L-Glutamine	53.5 ± 2.6	
	L-Glutamic acid	16.6 ± 2.10	
	L-Alanine	6.9 ± 1.0	
	L-Lysine	13.5 ± 0.9	
	L-Phenylalanine	11.1 ± 0.4	
	L-Proline	1.8 ± 1.1	

A similar result was found by Ghoora et al. (2020), in which a 120%, 127%, and 119% higher ascorbic acid content was found in microgreen stages of fenugreek (*Trigonella foenum-graecum* L.) as compared to their mature stage ($P \leq 0.05$). Microgreen stages of *Trifolium pratense*, *T. medium, Medicago sativa, M. lupulina, Onobrychis viciifolia, Astragalus glycyphyllos*, and *A. cicer* showed 0.6- to 3.2-fold higher Zn content compared to raw seed and sprout seeds of those legumes, which leads to significant higher nutritional composition in respect of mineral profiles study (Butkute et al. 2019).

9 Conclusion

Ever-increasing population has created a conflict for food and nutritional security and scientist are trying their best to find the best alternative which can provide a nutrient-rich, sustainable, and accessible source of fresh produce to communities around

the world. Rapid shrinkage of the land resources, healthy diet habits, lifestyle changes, etc. are also some of the factors which progressively resulted in increased interest of the people to include fresh fruits and vegetables and other kinds of green leafy vegetables as part of their daily diet to ensure adequate nutrition. In recent years, Microgreens have gained increasing popularity as food ingredients because of their high nutritional value and diverse sensorial characteristics. Leguminous microgreens, like other microgreens, are an emerging superfood, **g**aining attention for their nutritional density and culinary versatility. Leguminous microgreens, such as alfalfa, clover, lentil, chickpea, pea, mung bean, blackgram, cowpea, pigeon pea, fenugreek, horsegram etc., are particularly rich in protein, fiber, and essential amino acids, making them an excellent addition to plant-based diets. Additionally, growing leguminous microgreens requires fewer resources such as water, soil, and space compared to conventional agriculture along with they can be grown year-round in controlled environments, reducing reliance on seasonal produce and mitigating the environmental impact of long-distance transportation. Studying the benefits of underutilized leguminous microgreens and nutritional value and phytochemicals present at various growth stages of leguminous microgreens also give insights of how potential source of nutrition can be evolved in the coming future to combat the food and nutritional security of the world.

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Leguminous Microgreens and the Makeup of Their Nutrients

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Abbreviations

AOx Antioxidant activity

CVD Cardiovascular disease risk

FRAP Ferric reducing antioxidant power

HDL-C High-density lipoprotein cholesterol

LDL-C Low-density lipoprotein cholesterol

TC Total cholesterol

TPC Total polyphenols content

UVLs Underutilized vegetables and legumes

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1 Introduction

In recent years, nutrition has garnered more attention from the scientific community and the general public. These days, eating is not enough to stay alive; we also need to know what we put into our bodies to have the most significant possible existence for as long as possible. A healthy diet is now known to play a role in disease prevention and treatment, particularly in cases where an imbalanced, unhealthy, or inadequate diet is the underlying cause of health problems. Establishing an ideal human diet is currently one of the most contentious topics of conversation. What people eat and how they eat it varies significantly across the globe. A balanced diet should include all the essential nutrients for optimal nutrition (Butkutė et al. 2017).

The diet must supply:

1. Appropriate caloric intake from food sources to sustain metabolic and physical activities.

2. Food ingredients that have regulatory roles and rheological characteristics.

Maintaining a healthy nutritional balance is of the utmost importance. According to the FAO-WHO Group of Experts, these food suggestions should be followed: Calories from protein cannot be higher than 15%, carbs cannot be higher than 60% (with 3% coming from fibre), and fats cannot be

higher than 25%. The ratio of plant to animal products should be 75%. According to numerous epidemiological research, a plant-based diet lowers the risk of chronic illness. Plant-based foods that humans consume include roots, tubers, stalks, leaves, flowers, fruits, and seeds (Week and Production n.d.). Regarding the sheer quantity of species, the legume family ranks high. Legumes are a nutrition hotspot for good reason: they include protein, carbohydrates, fibre, vitamins, and microelements in sufficient amounts. Physically fractionating the grain into fundamental components like protein, starch, and fibre increases the value of pulses. These products can then augment other ingredients in cuisine, making it more nutritious. Still, a class of chemicals called antinutrients tends to impede the absorption of some nutrients when plant foods are eaten. Sometimes, these can harm the body's physiology or even be poisonous (for example, flatulence). Nevertheless, the importance of pulses to one's health is now more understood (Belhadj Slimen et al. 2021).

According to recent epidemiological studies, numerous antinutrients may help stave off cancer and heart disease if taken regularly. Pulses require further research into their bioactive and functional characteristics so that their use can be better understood. Legume seeds are rich in bioactive substances with a variety of biological functions. Some examples of glycosides are alkaloids, tannins, saponins, a-galactosides, lectins, a-amylases, vicine, and convicine. This means there are specialized ways to extract, determine, and quantify them. They have various physiological effects and do not manifest in all plants (Fig. 1). Vicia fib's pyrimidine glycosides, vicine, and convicine cause favism. Neuropathies, produced. Seeds are another example. Beans contain several bioactive compounds, including flatulence-causing a-galactosides. As a part of their defence mechanisms, plants use bioactive chemicals

to ward off harmful predators and extreme weather. Some of these compounds are reserve compounds that seeds store as energy for when they germinate (Carbas et al. 2023).

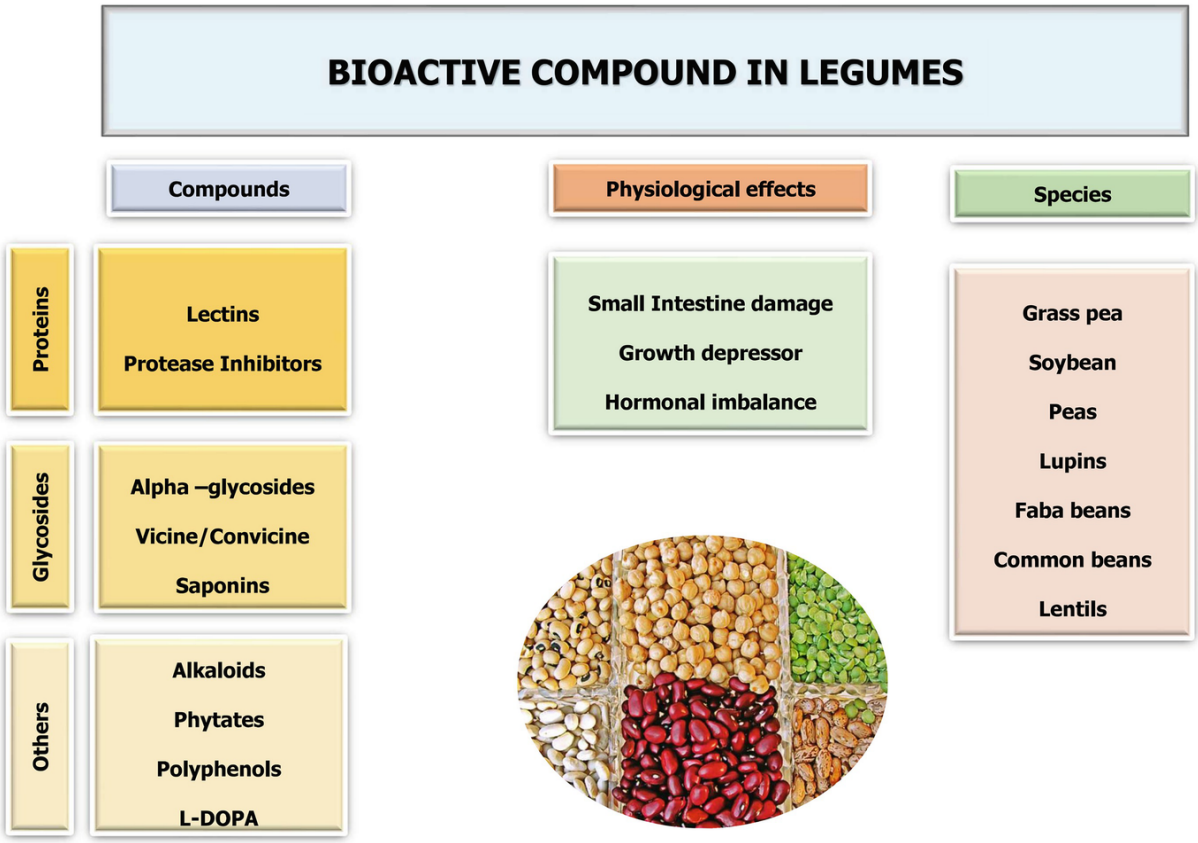


Fig. 1 Bioactive compounds in legumes

The evidence suggests that the chemical composition, concentration, duration of exposure, and interactions with other food components determine these compounds' beneficial or adverse effects. Thus, their impact on health determines whether they are considered antinutrients or nutrients. Knowing the types, quantities, and impacts of chemicals in food is, thus, essential. Much focus was on understanding how these compounds work and how they affect human nutrition, both positively and negatively. Currently, scientists are figuring out how different bioactive classes might work together and what practical

uses these molecules could have. This editorial contribution provides an overview (Danilčenko et al. 2017).

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2 Legumes in History

Legumes developed around 60 million years ago, according to extensive molecular study. Newer theories place the legume's origin in Laurasia, refuting earlier ones that said it evolved from western Gondwana. The Boreal hypothesis characterized the direct northward and subsequent southerly dispersal of legumes from the Americas. Legumes likely evolved in the northern area of the Tethys Sea and subsequently dispersed to other locations, according to subsequent testing of this concept. Long ago, humans began using legumes as a means of soil enrichment. Knowledge of its gastronomic merits was preserved in him. Their significance to the farming system has only grown since then (Garrido-Galand et al. 2021). Legumes were the focal point of the Romans' crop rotation practises. Legumes "reinvigorate" the soil, according to the Greek philosopher and botanist Theophrastus, and beans are manure, not a burden. Grain legumes were present from the beginning of grain domestication. Planting lentils (10,100–9700 cal BP), peas, chickpeas, and vetch (9900–9500 cal BP) yields convincing evidence in favour of the point of view. Crop rotation and mixed farming have been well-documented in India for millennia, with legumes, cereals, and other plants having human uses among them. Even as a staple food, legumes were highly regarded throughout that era. Archaeological evidence from ancient Egypt and Eastern Asia reveals that several legumes had practical uses. They used beans, alfalfa, peas, vetches, and soybeans. The history of legumes includes the following: the consumption of wild lentils in Greece around 11,000 BC, prior to their agricultural production; the discovery of carbonized seeds of certain legumes in Turkey during the Neolithic period

(7000–8000 BC); the cultivation of peas by lake dwellers in Switzerland (4000–5000 BC); the cultivation of soya beans in China (2000–3000 BC); the cultivation of beans and soya beans in the Americas; and the cultivation of staple crops in Asia (1000–2000 BC). In 1500 BC, lentils appeared in a few Cuneiform dishes (Reddy et al. 1983). Legumes of all kinds, adapted to various climates, were abundant throughout the Mediterranean. Despite its Chinese origins, soya beans have become a global phenomenon, earning the moniker “wonder crop” for the adaptability of their plant life. Each legume crop has its unique background, including where it originated, is distributed in the agroecosystem, and is used as food, fodder, and medicine (Singh et al. 2013).

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3 Influences on Legume Composition and Characteristics

There is various factor which can affect the composition and Characteristics of legume which is mention bellow like weather and fertilization, in below paragraph it is explain about the factor.

3.1 Weather Conditions

In many species of legumes, crop productivity is significantly affected by environmental changes, particularly abiotic stress, whereas quality might be positively or negatively affected. Plants undergo a cascade of physiological stress reactions in response to environmental stress, altering biochemical and enzymatic pathways that ultimately alter the chemical makeup of crops and, by extension, their quality when harvested. Species, exposure duration and intensity, and other interacting factors will determine the specific effects of stress. A rise of 25% in ozone and 22% in carbon dioxide concentrations, a 25% increase in salinity, and a 50% drop

in water availability are all factors that are likely to reduce the production of legumes and vegetables (Binou et al. 2022). In addition, we can expect a 4 °C increase in average temperature by the year 2050. All these things will majorly impact the global production, composition, and quality of vegetables and legumes. Raising temperatures during the reproductive stages of legumes has a negative effect, decreasing harvest yields and resulting in smaller seeds. Under heat stress, mineral and antioxidant content increases while fat content decreases. Hydric stress occurs when the chickpea crop experiences excessive daytime transpiration due to scorching weather. Drought stress lowers chickpea crop productivity in subtropical regions. However, stressed crops and their products have improved starch, lipids, and sensory characteristics, increasing nutritional composition and phenolic compounds. In response to heat and drought, modern plant breeding programmes focus on increasing crop quality while preserving yields and seed nutritional value. This may form a component of a more comprehensive suite of climate-savvy agricultural practices and technology (Bressani 1972).

3.2 Fertilization

Soil fertilization also affects legume grain quality and composition. Leguminous plant crops are advantageous because they are good biological nitrogen fixers. Nitrogen, one of the most crucial elements for plant growth, directly impacts harvest yields. Soil nitrogen fertilization benefits legume seed production and weight; one chickpea crop that received nitrogen fertilizer throughout the growth stage had a seed weight increase of 18.5% and a seed yield increase of 20%. Potassium (K) plays an essential function in withstanding water stress. Leguminous beans with lower protein contents and more significant concentrations of antinutrients are grown in potassium-deficient soils,

inhibiting the nitrogen (N_2) fixation mechanism. Sulfur (S) is another critical component in nitrogen fixation; when soil sulphur levels are low, legume yields and quality suffer. One benefit of sulphur fertilization is increased protein and amino acid levels; conversely, it might increase antinutrients like tannins (Adebo et al. 2022).

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4 Legume Seed Nutrition

The chemical makeup of legumes differs across seed varieties. Legumes typically have a high energy content, ranging from 330 to 374 kcal/100 g dry matter. Legumes contain anywhere from 20% to 35% protein, with the exact percentage varying widely across species. Compared to cereals, it has a high protein content and is an excellent source of lysine and sulphur amino acids like methionine, which are scarce in plant-based diets. While legumes add a lot to a diet when combined with starch-rich cereals like corn and other items, their nutritious value pales compared to animal proteins. Nevertheless, as explained in this chapter, thermal treatments might boost their nutritional value during conventional culinary preparation by inactivating the protease inhibitors (Ojiewo et al. 2019). The structural modifications that proteins undergo during processing make digestive enzymes more accessible and promote better protein metabolism. Furthermore, nutritional studies should account for the significant proportion of non-protein nitrogen (peptides, free amino acids, and other nitrogenous substances) measured as total protein, meaning the protein content is typically underestimated. A significant amount of this nitrogen is found in legumes. On the other hand, people with diabetes should eat legumes since the intestines better absorb complex carbohydrates and digest them more slowly than simple carbohydrates like sugar. Except for oilseeds, most

carbohydrates in legumes are starch (75–80%); peanuts have a third of their carbs from starch, and soybeans have almost no starch (Kaale et al. 2023). Legumes are a good source of dietary fibre, with the soluble fibre portion being significant (around 16–20%). The increased intestinal bolus aids in bowel movement and prevents constipation which beans contain, helps lower blood cholesterol levels, according to clinical trials and epidemiological studies. This is crucial information for lowering the risk of cardiovascular disease. Legumes have 1–4% fat. The exception is oilseeds, which average 18% fat for peanuts and 50% for soybeans. Health benefits include a lower CVD. The taste of a legume-based meal depends on how it is prepared. Seasoning cooked beans with salt and other ingredients may enhance their sodium content. Making healthful food requires several considerations. Legumes contain calcium (32–394 mg/100 g) and phosphorus (203–800 mg/100 g) (Sharma et al. 2021).

Legumes are rich in iron, a limiting mineral in diets that causes anaemia, a common health issue in children and women, especially during pregnancy. Magnesium, zinc, and calcium help prevent osteoporosis by mineralizing bone. Iron, magnesium, and zinc in legumes range from 1.6 to 6.3 mg per 100 g. Zinc is crucial in children's healthy development and immune system function. However, divalent cations (Ca^{2+}, Fe^{2+}, and Zn^{2+}) in legumes form insoluble complexes with phytic acid, which hinders their absorption and decreases their bioavailability. Iron from plant foods (non-heme iron) is less bioavailable than heme iron from animal-derived diets, even though legumes have a high Fe content. Also, phytic acid has a lot of phosphorus, so the body cannot use it. Legumes have a lot of different vitamins and are a good source of B vitamins like thiamine, which can range from 0.3 to 1.6 mg per 100 g, comparable to or even more significant than cereals. It has been found

that the processing has altered the content of these chemicals. According to various research, Flavonol glycoside concentrations rise in cooked chickpeas and germinated peas, beans, and lentils (Vaz et al. 2015). Regarding nutrition, most species of legumes are similar in composition. Variations in the quantities of phenolic chemicals cause the distinct colour of legumes. For example, white beans have a lower concentration of polyphenols and anthocyanins than other beans, which are more colourful. The high antioxidant capacity of these bean phenolic components has been supported by the fact that they may lessen the likelihood of developing chronic diseases like diabetes, cancer, and cardiovascular disease in the laboratory and living organism tests. Finally, research suggests that black beans, not white beans, are better at preventing long-term health problems. Various kinds of proteins, including those with storage and those without, as well as oligosaccharides, tannins, and alkaloids, are among the legume seeds' non-nutritional components. These components were formerly considered antinutrients due to their low metabolizable availability and potential for deleterious consequences if not processed before ingestion (Graham et al. 2001). Conversely, human nutrition aims for a balanced diet, but animal production prioritizes rapid development and weight increase. Legumes have focused much on α-galactosides and other non-nutritional factors for many decades. These indigestible oligosaccharides vary among legumes and can make up to 10% of the dry matter. "Antinutrients" has recently been redefined as bioactive or non-nutritional compounds due to their involvement in health-promoting effects in humans. The bacterial fermentation of the raffinose oligosaccharides (α-galactosyl derivatives of sucrose) in the colon causes gas production (H_2, CO_2, and CH_4), which in turn causes flatulence and osmotic diarrhoea, which are unpleasant abdominal

inflations caused by a deficiency of α-galactosidase (Muzquiz et al. 2012).

Nevertheless, because these compounds dissolve in water, they and other non-nutritional substances found in raw grains will stay in the soaking water if legumes are soaked for at least an hour before cooking. Sodium bicarbonate increases the permeability of the soaking water, which in turn improves the efficiency of soaking by partially solubilizing the cell walls. To partially remove α-galactosides, one might alternatively utilize germination or fermentation procedures. Recent research has linked these molecules to the benefits of dietary fibre, including normalizing intestinal transit, boosting lactobacilli and bifidobacteria colonies, and reducing N-nitroso compounds. The marketing of these oligosaccharides' centres on the idea that they can improve microbial health (Ebert 2014). Recent in vivo investigations have shown that legumes, specifically phytic acid (IP6), the monophosphate hexaphosphate ester and the principal source of phosphorus storage, can contribute anywhere from 3% to 5% of the total. Legumes also have prebiotic benefits and have immune-stimulant characteristics. Phytic acid is an effective chelating agent because of the six phosphate groups on the IP6 molecule; as a result, divalent cations such as Zn^{2+}, Fe^{2+}, Mn^{2+}, Mg^{2+}, and Ca^{2+} are less bioavailable. Phytic acid is, therefore, considered an inert substance. The production of insoluble metal-phytic complexes in the acidic intestinal environment restricts their absorption in animals and humans. IP6 can form phytate-protein complexes through its protein-binding capabilities. Digestion enzymes such as pepsin, trypsin, and α-amylase are inhibited by these complexes, which also affect the solubility and digestibility of protein in the diet (Cheng et al. 2019). The quantity of phytic acid that can be identified changes as a function of treatment circumstances

(time and temperature), pH, proteins, and cations in the molecule. While cooking does reduce some of this acid, the amount that can be detected fluctuates. Cutting down on IP6 is a promising strategy for improving mineral bioavailability. An enzyme called phytase is naturally present in seeds and catalyzes the hydrolysis of IP6 to inositol plus orthophosphate. This can be achieved through legume seed germination and fermentation (Keskin et al. 2022).

That is contained in foods. Phytic acid's ability to chelate iron makes it a powerful antioxidant that blocks the formation of hydroxyl iron radicals. As shown in recent research, when combined with iron in the colon, Phyto acid may aid in decreasing lipid peroxidation and hydroxyl radical formation. The subsequent decrease in the likelihood of colon cancer is possible. Inhibitors of enzymes and lectins have been defined as substances that hinder nutrition for many decades. It has also been found that IP6 may restrict the formation and spread of colon tumours in rats via increasing blood NK cell activity (Hedley 2000). According to certain studies, many animal models have shown growth inhibition and lower digestion of carbohydrates and proteins. Most legumes contain α-amylase inhibitors, which slow digestion and cause a drop in blood sugar levels after eating them. This affects how easily starch can be digested. The protease inhibitors account for 0.2–2% of the total protein weight in soluble legumes. Multiple studies in rats, chickens, and pigs have demonstrated that protease inhibitors, particularly those that inhibit trypsin and chymotrypsin, restrict growth, cause pancreatic hypertrophy, and may even reduce metabolizable energy. These inhibitors set off a cascade of events that increase pancreatic chymotrypsin and trypsin production. Their customary culinary treatments—soaking, heating, or under-pressure cooking—greatly diminish their activity due to their thermolabile nature. In recent years,

protease inhibitors have been associated with several positive outcomes, such as reduced inflammation, improved neurodegenerative and autoimmune diseases, and the prevention or suppression of tumour pathogenesis (Watson et al. 2017).

In addition, there is some indication that they could help with and even prevent obesity and hypertension. Lectins, a broad glycoprotein group, can reversibly attach to sugar residues on surface glycoproteins throughout the digestive tract (stomach, small and large intestine). Depending on the organ affected, the results could vary from an enlarged colon to slight intestinal hyperplasia. Additionally, lectins may halt tumour growth by bringing about cell death and autophagy in cancer cells. The precise composition of the phytosterols found in legumes varies from variation to variety; for instance, lupins can contain up to 53 mg/100 g of phytosterols. Most often found phytosterols include β-Sitosterol, campesterol, and D5-avenasterol. These secondary metabolites in plants reduce serum total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) and may increase HDL-C, which may affect obesity and atherosclerosis (HDL-C) (Voisin et al. 2014). Research has shown that phytosterols can reduce cholesterol absorption in the intestines by forming micelles with it instead. Most legumes contain saponins, including beans, peas, lupins, lentils, and chickpeas. Saponins are a class of chemicals that bind a carbohydrate moiety to a triterpenoid or steroid; these molecules are complicated and have multiple beneficial impacts on health. In the liver of mice, phytosterols may control genes involved in cholesterol metabolism, but in the intestines, they do not. Some research suggests that saponins may have anticancer effects by modulating the immune system. The literature also attests to saponins' lipidemic action. Most studies suggest they may reduce blood lipids by binding to cholesterol to form an insoluble molecule and preventing

its absorption in the intestines. Saponins may dissolve in the soaking, washing, and blanching supernatants and be lost during processing (Williams et al. 1994). An efficient heat processing procedure is essential for the food industry. Improves legume quality without lowering saponin levels. Lupins, peas, chickpeas, and lentils are the legumes most commonly found with alkaloids, but lentils and chickpeas have very modest amounts. These molecules may have several biological actions at deficient concentrations, mitigating the neurotoxicity that may otherwise cause unwanted side effects. Recently, Lupanine is an alkaloid of the lupin quinolizidine family that, according to research by it increases insulin secretion in response to glucose by acting directly on the ATP-dependent insulin receptor (Popoola et al. 2019). This manuscript demonstrated that K^+ channels improved glucose tolerance and insulin gene expression (Ins-1 gene) in rats who had been artificially hyperglycemic. Thus, lupanine could be helpful in the adjunctive management of diabetes mellitus (Semba et al. 2021).

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5 Untapped Bioactive Substances in Vegetables and Legumes

Figure 2 presents an illustration of the number of phytochemicals that can be found in legumes and vegetables both. The following are some examples of the substances that fall into this category: sugar alcohols, organic acids, lipids, nitrogen-containing compounds like amines, cyanogenic glycosides, glucosinolates, purines, and various nitrogen compounds; and finally, alkaloids like pyridine, betalain, indole, indolizidine, pyrrolidine, quinoline, and steroidal alkaloids (carotenoids). Most of the time, ultraviolet lights extract and analyze phytochemicals (Scarafoni et al. 2007).

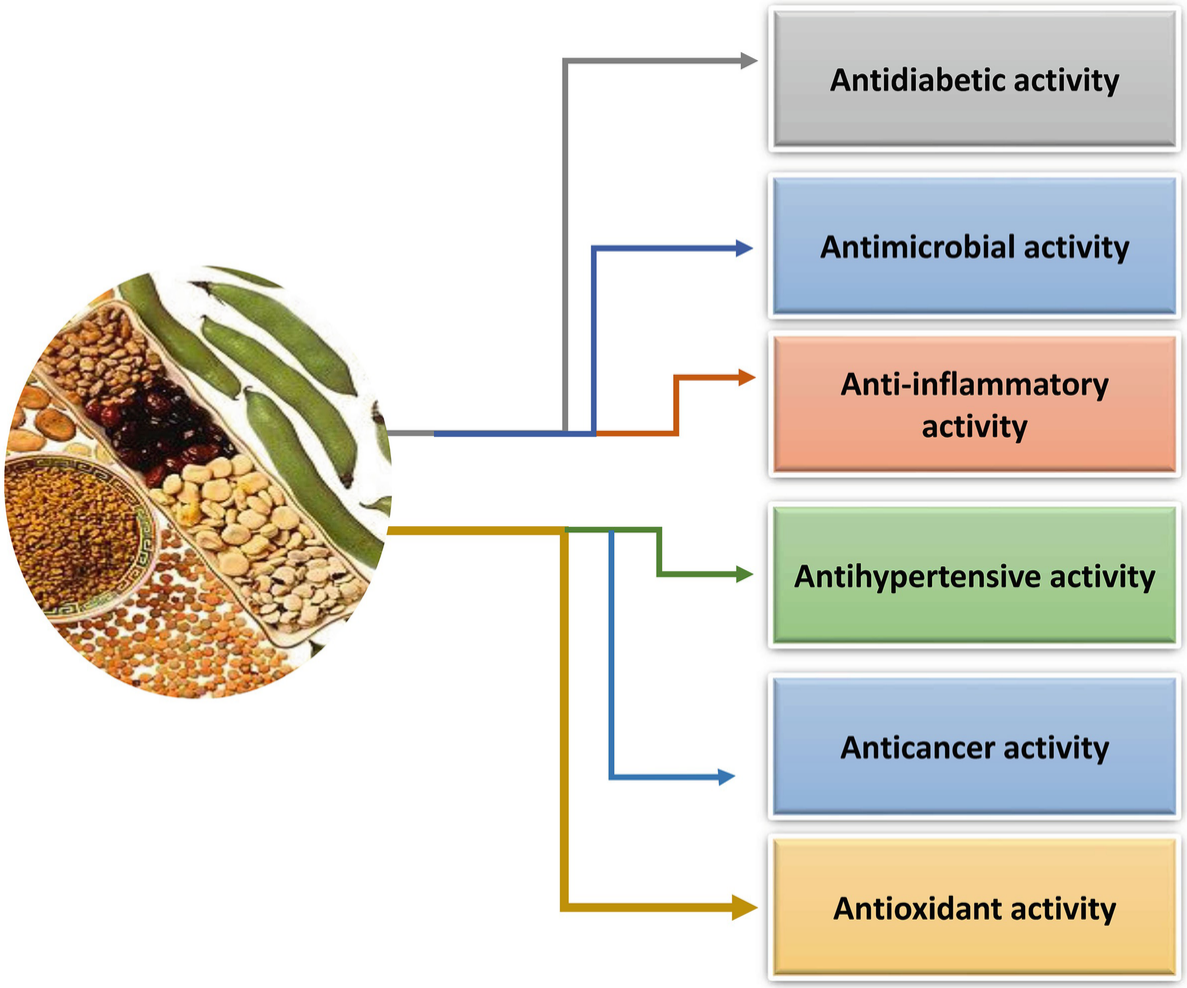


Fig. 2 Bioactive substances in vegetables and legumes

5.1 Polyphenols

A polyphenol is a chemical molecule with an aromatic ring and hydroxyl or hydrogen bonding groups. Flavonoids and nonflavonoids are the two primary categories of polyphenols. Xanthones isoflavones are all types of flavonoids. Other types of flavonoids include hydrolysable tannins, benzoic acids, acetophenones, phenylacetic acids, hydroxycinnamic acids, and benzophenones. Research shows polyphenols can modulate endogenous antioxidant enzymes, stabilize divalent cations, and scavenge free radicals. Flavonoids and phenolic acids are anti-carcinogenic and antimutagenic because they shield DNA

from free radicals, deactivate carcinogens, inhibit enzymes that promote carcinogenesis, and activate enzymes that detoxify xenobiotics. In vitro studies have shown that N-nitroso compounds formed by caffeic acid and chlorogenic acid can cause cancer and mutations. Some of the physiological impacts that tannins have been shown to have include modifying immune responses, lowering blood pressure, decreasing serum cholesterol levels, and speeding up blood clotting. It has been found that tannins, isoflavones, phenolic acids, and stilbenes have antibacterial properties (Bellucci et al. 2021).

Polyphenols are abundant in UVLs as well. The bitter gourd is rich in carotenoids, flavonoids, and phenolic substances. These acids are gallic, protocatechuic, gentisic, vanillic, chlorogenic, tannic, and caffeic and also contain kaempferol, myricetin, luteolin, and quercetin, among other carotenoids and flavonoids. Lastly, lycopene, zeaxanthin, ß-cryptoxanthin, lutein, and α-carotene are all present in bitter gourd. Some components found in Roseville are anthocyanins, flavonoids, sterols, Elphinidin, cyanidin-3,5-diglucoside, gossypicyanin, and anthocyanidin (Table 1). These results show that UVLs can be a fantastic way to get the extra polyphenols you need without changing your diet too much (Kumar et al. 2012).

Table 1 Phytochemicals in some underutilized vegetables and legumes

Common name	Botanical name	Major phytochemicals	References
Adzuki bean	*Vigna angularis*	Phenolics: procyanidin dimers, peonidin-3-rutinoside, malvidin-3-O-glucoside, myricetin 3-rhamnoside, quercetin glycosides, and catechin glycosides	Duranti (2006) and Iannetta et al. (2021)
Horse gram	*Macrotyloma uniflorum*	Kaempferol, myricetin, daidzein, and genistein are all examples of flavonoids.	Scarafoni et al. (2007) and

Common name	Botanical name	Major phytochemicals	References
			Amarowicz (2020)
Rice bean	*Vigna umbellate*	Daidzein, genistein are flavonoids. Some phenolic acids are gallic, protocatechuic	Renna and Paradiso (2020) and Keskin et al. (2022)
Stinky bean	*Parkia speciosa*	Quercetin, apigenin, kaempferol, luteolin, and myricetin are flavonoids. Polyphenols: quercetin, gallic acid, catechin, and ellagic acid Stigmasterol, and squalene are terpenoids.	Butkute et al. (2019) and Binou et al. (2022)
Water convolvulus	*Ipomoea aquatic*	Flavonoids include quercetin, apigenin, kaempferol, luteolin, and myricetin. Polyphenols: catechin, ellagic acid	Reddy et al. (1983) and Sinkovič et al. (2023)
Purslane	*Portulaca oleracea*	Kaempferol, myricetin, quercetin, portulacanones are flavonoids.	Bhat and Karim (2009) and Eswaranpillai et al. (2023)
Bitter gourd	*Momordica charantia*	Syringic, p-coumaric, gentisic, vanillic, chlorogenic, tannic, caffeic, ferulic, sinapic, and protocatechuic acids are phenolic chemicals. Flavonoids include apigenin, luteolin, kaempferol, epicatechin, quercetin, myricetin, and catechin.	Muzquiz et al. (2015) and Agnihotri and Rana (2021)
Roselle	*Hibiscus sabdariffa*	Plant acids: malic, tartaric, hydroxycitric, citric, and hibiscus. A class of anthocyanins that includes gossypicyanin, delphinidin-3-sambubioside.	De Ron (2015) and Ntatsi et al. (2018)

5.2 Terpenes

Terpenes are aromatic, naturally occurring chemicals that plants make from isoprene molecules. The number of isoprenoid units in a terpenoid structure determines their classification. Monoterpenes, sesquiterpenes, diterpenes, triterpenes, and tetraterpenes are the molecules that make up this class. These compounds have two to eight isoprenoid units. Terpenes have several biological and physiological effects. One effective anticancer medication is the diterpene lactone found in Andrographis paniculata. A well-liked antimalarial medication is sesquiterpene lactone, derived from the Artemisia species. A powerful anticancer, hepatoprotective, and anti-inflammatory medicine, cucurbitacin B is derived from the Cucurbitaceae family of plants (Momordica spp., Cucumis spp., Bryonia spp.). Terpenoid chemicals are stored in UVLs (Table 1). For instance, bitter gourd is rich in terpenoids like triterpenes, saponins, and phytosterols (Table 1). Purslane supposedly includes a variety of chemicals, including portulenes, monoterpene glycosides, Portulosides A and B, and others many phytosterols (Table 1) (Balázs et al. 2021).

5.3 Alkaloids

Alkaloids are a class of chemical compounds that are characterized by the presence of basic nitrogen atoms. Many alkaloids have helpful clinical applications, ranging from analgesics to cancer chemotherapeutics, and they all have pharmacological solid bases. For instance, the periwinkle plant in Madagascar is the source of the vinca alkaloids vinblastine and vincristine (Catharanthus roseus). Some alkaloids have been found to have antimalarial properties; they include terpenoids, indole, bisindole, quinoline, and isoquinoline. Evidence suggests that the pyridine alkaloids arecoline (found in areca nut) and the isoquinoline alkaloid morphine (found in opium poppy) help

improve symptoms of neurodegenerative illnesses, including Alzheimer's and schizophrenia, respectively (Didinger and Thompson 2021; Iannetta et al. 2021).

5.4 Volatiles

Essential oils and volatiles come from various plant and animal sources, including terpenoids, amino acid derivatives, alcohols, esters, aldehydes, ketones, alkanes, and alkenes. The cosmetics and perfume industries have long used essential oils, which have long been associated with various human biological processes. For example, a mouse model has shown that the lipophilic sesquiterpene ßcaryophyllene has potent anti-inflammatory effects. The humulene sesquiterpene zerumbone induced G2/M cell cycle arrest and death in Jurkat cancer cell lines using the mitochondrial route, demonstrating anticancer and chemoprotective properties. Countless other plant volatiles have shown antiparasitic, insecticidal, fungicidal, bactericidal, and virucidal effects. Some volatile chemicals in roselle include eugenol, furfural, 2-ethylfuran, hexanal, and the terpenes 1,4-cineole and limonene (Table 1) (Urbano et al. 2007).

5.5 Lectins

Lectins are glycoproteins that attach sugar and are present in many plant foods, but they are especially abundant in legumes. They do not trigger particular reactions and have nothing to do with the immune system. Antinutrients are proteins that impede nutrient digestion and absorption by binding to sugars on the surfaces of cells in the gut wall. The proteolytic digestion, heat denaturation, and immunomodulatory, cytotoxic, antibacterial, and insecticidal properties of legume lectins are just a few of their many biological functions. Lectins from plants are well-known as natural insecticides due to their capacity to attach to insects' stomachs and kill a broad range of insect

species. Insect larvae that consume lectins face several problems, such as delayed maturation, reduced size and weight increase, disruption of female fertility, lower rates of pupation and adult emergence, lengthened overall development time, and mortality in sporadic cases. As potential insecticides, these lectins have been genetically engineered into various crops. There is evidence that some lectins have anticancer properties, including inhibiting the proliferation of specific cancer cells and triggering cell death in others. Research has demonstrated that lectins can suppress the growth of many bacteria and fungi, indicating their antibacterial action. Two other legumes, Canavalia ensiform is and Lathyrus cicero, were also shown to contain lectins (Saleh et al. 2013).

5.6 Glucosinolates

There are a lot of glucosinolates and thioglucosides in Brassicaceae plants, which include cabbage, cauliflower, brussels sprouts, and broccoli. These chemical compounds, formed from glucose and an amino acid, are part of a class that includes sulphur and nitrogen. The thioglucoside glucohydrolase enzyme is present in plants with glucosinolates (myrosinase). The parent glucosinolate undergoes hydrolysis when it comes into touch with myrosinase in a water-containing environment. An aglycone group, glucose, and sulphate make up the hydrolysis byproducts. Glucosinolate structures stabilize the aglycone molecule. These chemicals are potent anticarcinogens and protect plants from illnesses and weeds. For instance, specific glucosinolates suppress plant pathogen growth. We have found luciferin, glucoerucin, glucoheirolin, and glucotropaeolin. Research also shows that alkyl and aryl glucosinolate hydrolysis products harm Salmonella typhimurium. The food industry uses alkyl isothiocyanates as a preservative because of this. The bioactive byproducts of glucosinolate hydrolysis show promise as naturally

occurring insecticides against nematodes and other soil-borne pests. It has been found that glucosinolates and the byproducts of their hydrolysis possess antimutagenic and chemopreventive properties. Glucosinolates were also found in underappreciated plants (Iannetta et al. 2021).

5.7 Organic Acids

There are a lot of organic acids in underappreciated vegetables; for example, roselle has tartaric acid, malic acid, hibiscus acid, citric acid, and hydroxy citric acid (Table 1). In this context, hydroxycitric acid is an antiobesity medication that is said to be present in brindle berries and kokum fruits. Another possible source of hydroxy citric acid is the calyx of flowers, known as roselle calyces. Because roselle calyx extract contains tartaric, malic, and citric acid, it may also have antioxidant properties (Rubiales and Mikić 2015).

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6 Composition of Bio Active Content Compound in Legumes

In bellow paragraph showing the composition of content like Carbohydrates, Protein, Chlorophyll and ascorbic acid estimated amount in different-different vegetables and fruits. These are essential content which play an important role in health benefits. These are also play a very vital role in different-different disease.

6.1 Composition of Carbohydrates Content

See Table 2 for sprout and microgreen carbohydrate content. The lowest carbohydrate content was 7.27 mg/g in mung bean sprouts, followed by 10.50 mg/g in sorghum and 9.17 mg/g in wheat. The sprouts of cowpeas had the fewest carbs (4.73 mg/g). The sequence was as follows: sorghum came first, followed by wheat, mung beans,

fenugreek, horse gramme, and last, cowpea. The carbohydrate values of sprouts show a solid connection ($F = 1170.757$; $P < 0.001$) (Table 2) (Reddy et al. 1983).

Table 2 Carbohydrate estimation for sprouts

Sprouts	Carbohydrate value (mg/g)
Fenugreek	6.83 ± 0.03d
Mung bean	7.27 ± 0.03c
Cowpea	4.73 ± 0.03f
Horse gram	5.73 ± 0.03e
Wheat	9.17 ± 0.03b
Sorghum	10.50 ± 0.10a

The significance of this data lies in comparing the values of a specific parameter (likely nutritional or chemical content) across different food items. The statistical grouping (a, b, c, d, e, f) indicates that Sorghum has the highest value, while Cowpea has the lowest, highlighting differences in the parameter's concentration among these foods. This information can be useful for selecting food sources based on the desired nutrient or property.

6.2 Composition of Protein Content

The protein composition of sprouts and microgreens in Table 3, respectively. At 5.83 mg/g, mung bean sprouts had the highest protein level, followed by 5.73 mg/g of fenugreek and 5.63 mg/g of horse gramme sprouts. With 2.40 mg/g of protein, sorghum sprouts had the lowest protein level. In this sequence, mung bean came first, followed by fenugreek, horse gramme, cowpea, wheat, and sorghum. A strong correlation exists between sprouts' protein content and their nutritional value ($F = 2087.400$; $P < 0.001$) (Table 3) (Binou et al. 2022).

Table 3 Estimation of proteins in sprouts

Sprouts	Protein content (mg/g)
Fenugreek	5.73 ± 0.03ab
Mung bean	5.83 ± 0.03a
Cowpea	2.77 ± 0.03c
Horse gram	5.63 ± 0.03b
Wheat	2.43 ± 0.03d
Sorghum	2.40 ± 0.06d

Fenugreek: 5.73 ± 0.03ab (same as Horse gram, different from Mung bean and Cowpea)Mung bean: 5.83 ± 0.03a (significantly higher than Fenugreek and Horse gram, and much higher than Cowpea)Cowpea: 2.77 ± 0.03c (significantly lower than all others)Horse gram: 5.63 ± 0.03b (significantly lower than Mung bean but higher than Cowpea)

6.3 Composition of Chlorophyll

Table 4 and Supplementary Table 2 show sprout and microgreen chlorophyll. Chlorophyll a, b, and chlorophyll in sprouts were 3.73–0.53 mg/mL, 1.63–0.00, and 5.37–0.53. Mung bean sprouts have 5.37 mg/mL total chlorophyll, 3.73 mg/mL a, and 1.63 mg/mL b. Horse gramme sprouts had the lowest chlorophyll a, b, and total chlorophyll at 0.53 mg/mL. A substantial association exists between chlorophyll ($F = 1385.786$; $P < 0.001$), chlorophyll b ($F = 507.200$; $P < 0.001$), and total chlorophyll ($F = 1288.498$) (Adebo et al. 2022).

Table 4 Estimating sprout chlorophyll

Sprouts	Total chlorophyll (mg/ml)
Fenugreek	3.13 ± 0.07b
Mung bean	5.37 ± 0.03a
Cowpea	1.60 ± 0.06c
Horse gram	0.53 ± 0.03e

Sprouts	Total chlorophyll (mg/ml)
Wheat	1.50 ± 0.06c
Sorghum	1.07 ± 0.04d

Fenugreek: 5.73 ± 0.03ab (same as Horse gram, different from Mung bean and Cowpea)Mung bean: 5.83 ± 0.03a (significantly higher than Fenugreek and Horse gram, and much higher than Cowpea)Cowpea: 2.77 ± 0.03c (significantly lower than all others)Horse gram: 5.63 ± 0.03b (significantly lower than Mung bean but higher than Cowpea)

6.4 Composition of Ascorbic Acid

Tables 5 indicate sprout and microgreen ascorbic acid concentrations. Vitamin C levels in sprouts ranged from 1.63 to 0.03 mg/g. The table indicated that wheat sprouts had 0.03 mg/g ascorbic acid and horse gramme sprouts 1.63 mg/g. Ascorbic acid levels in sprouts are significantly correlated ($F = 432.667$; $P < 0.001$) (Rubiales and Mikić 2015).

Table 5 Estimation of ascorbic acid content in sprouts

Sprouts	Ascorbic acid (mg/g)
Cowpea	1.43 ± 0.03b
Fenugreek	0.33 ± 0.03d
Horse gram	1.63 ± 0.03a
Mung bean	0.57 ± 0.03c
Sorghum	0.07 ± 0.03e
Wheat	0.03 ± 0.03e

Fenugreek: 5.73 ± 0.03ab (same as Horse gram, different from Mung bean and Cowpea)Mung bean: 5.83 ± 0.03a (significantly higher than Fenugreek and Horse gram, and much higher than Cowpea)Cowpea: 2.77 ± 0.03c

(significantly lower than all others)Horse gram: 5.63 ± 0.03b (significantly lower than Mung bean but higher than Cowpea)

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7 Legumes and Their Associated Health Benefits

Researchers are increasingly interested in the health advantages of legumes, and this section aims to emphasize those benefits. Eaten alone or alongside other legumes, beans lessen the risk of diabetes, cardiovascular disease, and various malignancies. Research has demonstrated that legumes' polysaccharides, proteins, and bioactive compounds have different physiological effects. Researchers meta-analyzed a prospective cohort study to assess if legumes increase cardiovascular disease or other death risks. The findings demonstrated that a reduced risk of mortality from all causes is connected with high consumption of legumes. Furthermore, the data do not indicate that the group that regularly eats beans has an elevated risk of death from cardiovascular disease (Arbab Sakandar et al. 2023).

7.1 Antidiabetic Activity

Persistent hyperglycemia causes chronic difficulties in the body's metabolism; this is the hallmark of diabetes mellitus, an endocrine system metabolic illness. The three main types of diabetes are gestational, type 2, and type 1. UVL phytochemicals have been demonstrated to fight diabetes. From bitter gourd, produce charantin (Stagnari et al. 2017).

7.2 Anticancer Activity

Cancer killed 9.6 million people and caused 18.1 million new cases in 2018. One in five men and one in six women

will develop cancer, and one in eight men and one in eleven women will die from it. Nearly 43.8 million people will survive cancer within 5 years after diagnosis. Chemotherapy is a part of systemic therapy that can have some unintended consequences. An increasing amount of evidence indicates that plant-based bioactive compounds can be potent cancer fighters. There is mounting evidence that phytochemicals produced by UV light sources have potential cancer treatments (Duranti 2006).

7.3 Antimicrobial Activity

Research into phytochemicals with antibacterial capabilities that are safe, effective, and selective has long been lucrative due to the high prevalence of microbial infections as a threat to human health. Protocatechuic acid (phenolic) and roselle calyx extract were tested in vitro against methicillin-resistant Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Acinetobacter baumannii. Although both drugs inhibited all bacterial pathogens, protocatechuic acid showed a broader inhibition zone and lower MIC than roselle calyx extract. Horse gramme extracts kill E. coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Bacillus subtilis. These include D-ribose, D-xylose, D-mannose, D-galactose, and D-glucose. The antibacterial characteristics of phytochemicals exposed to UV radiation make them a promising treatment (Bohra et al. 2015).

7.4 Antihypertensive Activity

People with hypertension, characterized by consistently high blood pressure in the arteries, have the most significant chronic health problems. Several serious health problems, including heart disease, stroke, peripheral artery disease, dementia, chronic kidney disease, eyesight loss, atrial fibrillation, and heart failure, can be precipitated by persistently high blood pressure. An estimated 7.5 million

people die each year from hypertension, accounting for almost 12.8% of all fatalities on Earth. In recent decades, there has been a meteoric rise in the usage of phytochemicals found in herbal plants and medicinal herbs as a treatment. For instance, dried roselle (Hibiscus sabdariffa) calyces are commonly used to make a drink that helps with hypertension, and several UVLs are pretty effective in this fight (Sparvoli et al. 2015). Systolic blood pressure dropped 1.2%, and diastolic blood pressure dropped 10.7% after drinking roselle calyces' tea. A standardized extract was tested for effectiveness and safety on human volunteers and patients with mild to moderate hypertension. The results showed a decrease of over 10% in systolic and diastolic blood pressure. Herrera-Arellano conducted randomized, double-blind, lisinopril-controlled clinical studies on hypertension patients using a standardized herbal medicinal preparation of roselle; the results showed that the antihypertensive action was caused by diuretic activity (Upadhyaya et al. 2011).

7.5 Antioxidant Activity

Various reactive species, including free radicals and non-radicals, are produced in the body through metabolic reactions. These organisms can originate from nitrogen or oxygen. Proteins, DNA, and lipids are among the macromolecules they target, and their attacks injure cells and tissues. Various chronic illnesses, including cardiovascular issues, diabetes, cancer, and rheumatoid arthritis, are also caused by oxidants. Plant pigments, volatile oils, flavonol, terpenoids, and phenolics are antioxidant phytochemicals that help manage cellular oxidant levels. UVLs' phytochemicals are potent antioxidants (Barman et al. 2018). Figure 3 shows protocatechuic acid (phenolic acid) from Hibiscus sabdariffa blossoms protecting rat hepatocytes from tert-butyl hydroperoxide-induced cytotoxicity and genotoxicity

in a primary culture. This extract may have preserved cells by scavenging free radicals. The roselle-derived chemical delphinidin-3-glucoside, often known as anthocyanin, exhibited potent antioxidant properties components, including overacting, quercetin, kaempferol, rosmarinic acid, chlorogenic acid, and caffeic acid, exhibited potent antioxidant properties (Chibarabada et al. 2017).

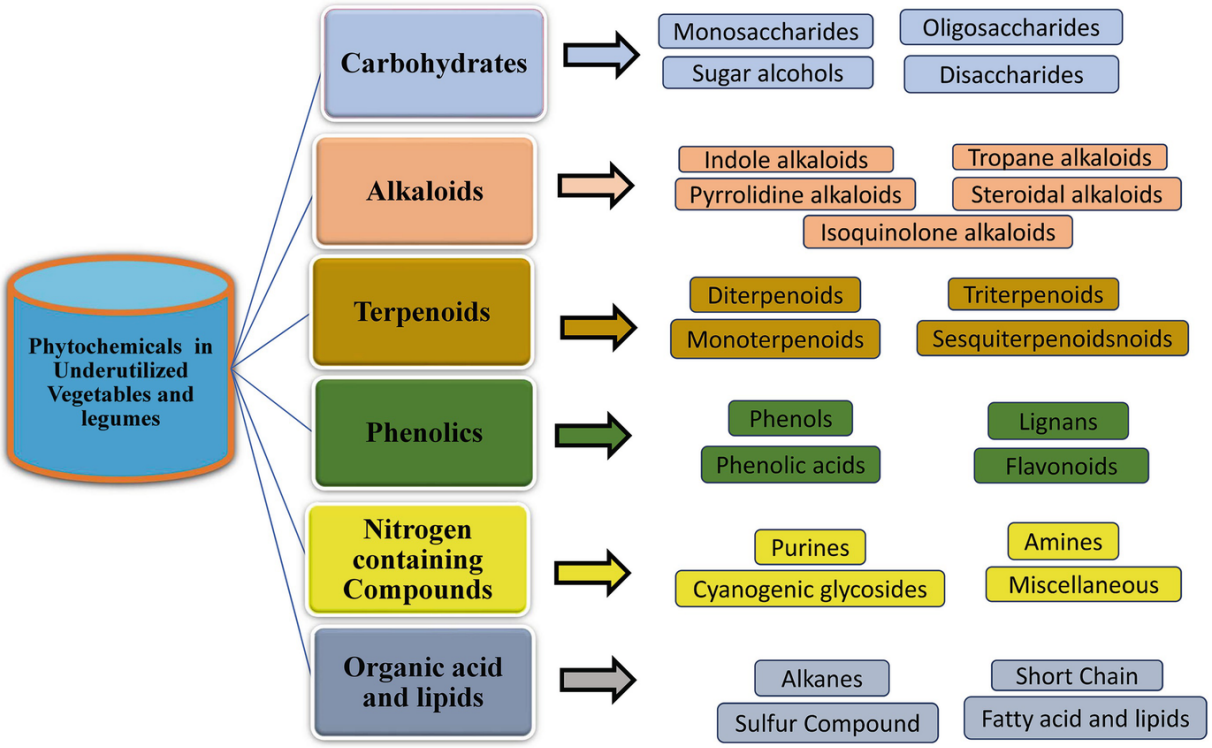


Fig. 3 Associated health benefits with legume

7.6 Hepatoprotective Activity

The liver is an essential organ that aids in the metabolism of lipids and carbohydrates and is responsible for the release of bile. Many potential chemical and biological factors might cause hepatic disorders, but eating a diet full of phytochemicals can help fight them. Evidence shows that phytochemicals found in underappreciated veggies can protect against liver damage. Roselle flower extracts protected primary hepatocytes against oxidative stress in

rats. The phenolic protocatechuic acid extracted from rose lilies protected hepatocytes from the cytotoxicity and genotoxicity caused by tert-butyl hydroperoxide. Among the processes linked to this characteristic is the elimination of free radicals. It was also shown that protocatechuic acid helped rats recover from liver damage caused by lipopolysaccharide (Abbas and Ahmad 2018).

7.7 Anti-inflammatory Activity

The body's complicated biochemical reaction to intruders, injury, and irritants is inflammation. Research shows that controlling inflammatory cytokines such as nitric oxide, interleukins, tumour necrosis factor-alpha, and interferon-gamma reduces inflammation. UVLs' anti-inflammatory properties come from phytochemicals. In Fig. 3, purslane alkaloid oleracone reduces inflammation in lipopolysaccharide-stimulated macrophages (Singh and Singh 1992).

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8 Legume Plants in Animal Production

Domestic sources of protein, such as sweet lupines (Lupinus), peas (P. sativum), and field beans, are being used more and more by slaughter animal breeders and producers (V. faba). According to scientific studies and production practice, moderate levels of legume seeds in animal diets do not negatively affect essential production qualities. However, since the quality of commercially available protein feedstuffs constantly fluctuates, studying the effects of different legumes on animal output, body composition, and meat quality (meat, milk, eggs) would be prudent. Modern plant breeding has made it possible to feed monogastric animals a variety of domestically grown seeds, including those of lupine (L. et al.), pea, field bean,

rape (cake or extracted meal), and soybean (G. max.) (Hulse et al. 1977). Due to antinutrients, the seeds of peas, lupine, and field beans cannot be used as animal feed. They are still used in animal feed, albeit in smaller quantities, even though they can limit the usage of nutritional components and can negatively impact animal health. Phytates, tannins, alkaloids, and proteolytic enzyme inhibitors are some of the antinutrients found in legume seeds. Technological treatments such as dehulling, extrusion, toasting, micronization, soaking, or fermentation can also increase the use of legume seeds for feed production (Sparvoli et al. 2016).

Many local, national, and international labs have studied the efficacy of feeding raw and cooked legume seeds to different animals, including cattle. Model animal studies and a review article both attest to the usefulness of the protein above meals as partial soybean meal alternatives. Pigs and other agricultural animals have also been the subjects of extensive research. Based on the results of these studies and prior experiments, it can be concluded that using legume seeds instead of genetically modified soybean meal did not hurt the fattening and slaughter parameters of the cattle species tested (Zhang et al. 2019).

Legume seeds can impact the quality of farm animal products. We performed the investigations. Fatteners' meat quality was not significantly different between groups given feed combinations with different proportions of yellow, blue, and white lupine (L. et al.) and field bean (V. faba) compared to groups fed diets without these additions. There was no discernible change in the milk's chemical makeup when dairy cows' diets included pea seeds (P. sativum). Tested the hypothesis that white lupine seeds improved rabbit meat quality. After doing the math, they found that MUFA levels were higher and SFA and PUFA levels were lower. In addition, layer chickens whose diets included blue and yellow lupine seeds had lower amounts

of saturated and monounsaturated fats and higher levels of polyunsaturated fatty acids in their yolk samples (Duc et al. 2015).

Consequently, turkeys fed a diet that included yellow lupine showed a shift in their breast muscle PUFA composition, possibly increasing PUFA content and decreasing SFA content. Researchers fed broiler chickens a diet that included legume seeds (such as Lupinus, Pea, or Field Bean; Lupinus, P. sativum, V. faba) and found that the chickens' breast and thigh muscles had different chemical compositions. Interestingly, the researchers also claimed that the feed mixtures used in the experiment did not negatively impact the meat quality (Foyer et al. 2016).

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9 Unconventional Goods That Include Beans

Legumes' physicochemical characteristics and food processing conditions dictate their functioning and application in food items. Table 6 summarises the solutions for the nutritional, functional, and sensory problems that arise when using legumes in food items. Because of their effects on sensory qualities, fibre, colour, and flavour are essential considerations in many applications. Pasta and baked goods containing varying amounts of beans have been the subject of numerous research aimed at assessing sensory properties and, by extension, customer approval. According to multiple studies, baked items made with 10% lentil, pea, or chickpea flour were more palatable in sensory assessments than wheat products. This includes baked rolls, biscuits, bread, and crackers. Enhanced quantities of lentil and pea flours were observed (Vaz Patto et al. 2015).

Table 6 Problems with nutrition, functionality, and taste caused by foods that include or substitute legumes

Agricultural product	The legume and the proportion incorporated	Sensory, nutritional, and functional obstacles	References
Non-GLUTEN crackers	Completely chickpea (100%)	Three to six times the amount of iron	Dhaliwal et al. (2020) and Arbab Sakandar et al. (2023)
Crisp biscuits	Pea (10%)	Wheat cracker biscuits did not vary significantly on any sensory characteristics.	Foyer et al. (2016)
Bread	Chickpea flour (10%)	Increased loaf mass overall, there were no discernible variations in acceptance when using wheat bread.	Sharma (2021)
Biscuits	12% bean flour followed by 24% bean flour	Protein content increased by 14% and the percentage of essential amino acids by 38%. A 26% increase in protein and a 35% increase in critical amino acids	Ferreira et al. (2021) and Iannetta et al. (2021)
Bread	12% lentil flour 24% lentil flour	Dietary fibre increased by 18%, total phenolic content by 57%, lignans by 96%, and free radical absorbing phytonutrients by 66%. Dietary fibre increased by 24%, total phenolic content by 61%, lignans by 97%, and free radical absorbing phytochemicals by 64%.	Week and Production (n.d.) and Duodu and Apea-Bah (2017)
Crackers	Broad bean flour comprises 40% green pea flour, 40% yellow pea flour, and 40% yellow pea flour.	Dietary fibre increased by 9.4%, total phenolic content by 20%.	Butkutė et al. (2017) and Rehman et al. (2019)

10 Conclusion

In this book chapter, we looked at the possible nutritional and health advantages of eating legumes. Legumes’

elevated levels of carbohydrates, proteins, dietary fibre, vitamins, minerals, and bioactive chemicals are mostly responsible for these benefits. In the large intestine, resistant carbs (such as dietary fibre, resistant starch, and oligosaccharides) have favourable prebiotic effects and contribute to gastrointestinal function. Their mineral and vitamin content, on the other hand, may help avoid illnesses caused by deficiencies, and their hydrolysed proteins may create peptides with biological activity. It indicates that the presence of bioactive chemicals in beans has a significant impact on the prevention of cardiometabolic risk. The nutritional content of legumes is enhanced by processing, which is necessary prior to intake. Because they are so beneficial to human health, legumes are attracting more and more attention and funding in recent years. Consequently, there has been a surge in interest in using beans in functional food recipes. The goal of this plan is to improve the global diet by boosting the intake of legumes. There is a worldwide epidemic of obesity and chronic malnutrition, and one potential therapeutic option might be the potential impacts of eating legumes. Legumes are crucial for achieving the goals of ending world hunger, promoting healthy eating habits, and protecting the environment, as stated by FAO-UN. Nutritious meals should be consumed immediately.

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Trailing the Prospects of Leguminous Microgreens as Rising Functional and Future Foods

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1 Introduction

In today's era, a healthy diet has become one of the most challenging and yet an important part of human life. The quest is not to eat just for the survival, but to be cognizant of functional and nutritional importance of food (Muzquiz et al. 2012). Organic, healthy and functional foods embrace a broad range of nutrients mainly characterized by diverse bioactive components which effectively promote health and prevent diseases beyond basic nutrition (Shahidi 2004). Such complex attributes are characteristic of legumes which contain high carbohydrates, minerals, proteins contents and a plethora of health promoting phytochemicals.

Legumes basically belong to the family *Fabaceae,* which is one of the largest families with over 18,000 species classified into 650 genera of flowering plants. The family Fabaceae forms a paramount component of nearly all terrestrial biomes in all continents (except Antarctica) (International Legume Database). Leguminous grains or pulses are one of the most valuable components of staple foods owing to their rich nutritional values, abundant minerals and secondary metabolites (Bouchenak and Lamri-Senhadji 2013). The results of archaeological researches imply that along with pulses, some perennial legumes have also been consumed as edible as well as medicinal plants since ancient times (Butler 1995; Weide et al. 2017). *Astragalus*, *Trigonella*, *Melilotus*, *Trifolium* and *Medicago* are certain species which are believed to be consumed as human food resources since ancient times. Conventionally, many herbaceous perennial legumes are used as highly nutritive fodder crops. However, the increasing interest in under-utilized plant species for food mainly arises from the findings and promotion of nutritionally relevant attributes (Dini et al. 2012). For centuries, their young leaves, plants, flowers and seeds have been used in phytotherapy as well as food (Ionkova 2008; Bora and Sharma 2011; Łuczaj 2012; Bouchenak and Lamri-Senhadji 2013). Numerous investigations on the distribution of secondary metabolites in perennial legume species, mostly in *T. pratense* and *M. sativa*, were focused on plants' aerial parts (Saviranta et al. 2008; Malisch et al. 2015). More recently, studies have demonstrated eminent proximate and phytochemical profiles as well as antimicrobial and antioxidant properties of aerial plant parts of seven temperate perennial species including, red clover, zigzag clover (*T. medium* L.), alfalfa, black medic (*M. lupulina* L.), sainfoin (*Onobrychis viciifolia* Scop.), liquorice and Cicer milkvetches (*Astragalus glycyphyllos* L., *A. cicer* L.) (Butkutė et al. 2017a, b, 2018a, b, c).

A plethora of literature is already available regarding the leguminous crops defending their phytochemical, functional exceptionally nutritional characteristics. Interestingly, a new

class of recently introduced functional foods called *microgreens*, has been added into the very options of superfoods. A number of studies corroborating the higher content of phytochemicals including isoflavones, rich mineral composition and other antioxidants in leguminous microgreens are available. The chapter summarises almost all the aspects of leguminous microgreens and collates the studies performed so far in the area of leguminous microgreens.

2 Leguminous Microgreen as an Indispensable Part of Human Diet

Microgreens are basically the germinating seedlings of many cereals, vegetables, grains and even flowers. These consist a pair of new cotyledonous leaves, a short delicate stem and root. The microgreens are short and tender greens generally harvested between 7 and 21 days after germination depending on the stage of growth. These little seedlings are becoming well known for their nutritional advantages in addition to their subtle tastes, colour and textures. These can be harvested and used as garnishing and visual components in various cuisines to complement the flavour, colour and texture. Although the precise nutritional value varies depending on the species of microgreens. The most common forms include broccoli, kale, radish, arugula, sunflower, etc. Out of a broad range of microgreens' variety, the leguminous microgreens are one of the least studied microgreens till date.

Leguminous microgreens have a plethora of health benefits as they contain some eminent bioactive compounds. The demands on dietary meals have expanded in line with the population's growth. The cultivation of food is becoming highly dependent on the use of chemical pesticides and fertilizers which cause deleterious impact on the well-being of environment. Higher reliability rates on such chemicals are exponentially increasing with increase in population and the hunger index globally. Malnutrition with nutritional insecurities is one of the usurping factors rising due to population overgrowth particularly in the developing nations. Malnutrition rates are higher in Asia and Africa than in other continents wherein, the prevalence of undernourishment has increased from 7.9% to 9.0% (from 2019 to 2020) in Asia. The 20.2% of African countries marked the undernourishment which was in line with 2021. In such an ever-rising nutritional demands, the microgreens are introduced as a leading candidate as far as plant-based superfoods are concerned. These are becoming highly popular from a dietary perspective

for being super rich in phytochemicals. Phyto-active compounds like chlorophyll, beta-carotene, lutein, polyphenols, glucosinolates, poly-sterols and minerals like magnesium, Potassium, Phosphorus, and Calcium are extensively present thereby adding to the nutritional and health-related functional aspects in microgreens (Petropoulos et al. 2021).

Legumes have always been an important diet component globally owing to the higher concentrations of dietary fibres, proteins and carbohydrates to make it a high-energy source food (Bubelová et al. 2018). Moreover, legumes are also rich in secondary metabolites, such as flavonoids, carotenoids, antioxidants, and chlorophyll. Microgreens of small-seeded legumes are newly introduced promising source of ingredients for fortification of staple foods with bioactive compounds, minerals and nutrients. The supplementary compounds that are generally not synthesized in the human body, have the potential to counter the nutritional deficiencies (Klopsch et al. 2018). Not only consumption of legumes provides rich nutrients to our body but also prevent the degenerative diseases, such as cancers, all thanks to the rich supply of bioactive compounds they ensure. Some legume microgreens of chickpeas, pea shoots, beans and lentils have been exploited to harness the best nutritional bioactive components. Some of the salient nutritional and functional benefits of leguminous microgreens includes, the easier cultivation, even in constrained space. This feature makes them handy for home cultivation and potentially a value-added and sustainable food production (Fig. 1). The total phytochemical content is many folds higher as compared to the wholesome sprouts or seeds thereby making them as potent source of functional compounds like minerals isoflavones, and phytochemical compounds. Another beneficial aspect deals in the therapeutic attributes of leguminous microgreens for having anti-cancer, anti-microbial, anti-depressant properties, etc. Furthermore, the versatility in culinary uses to upload the cuisines with vibrant taste and flavours thereby making them making them

a flexible component in salads, sandwiches, wraps, tacos, smoothies, etc.

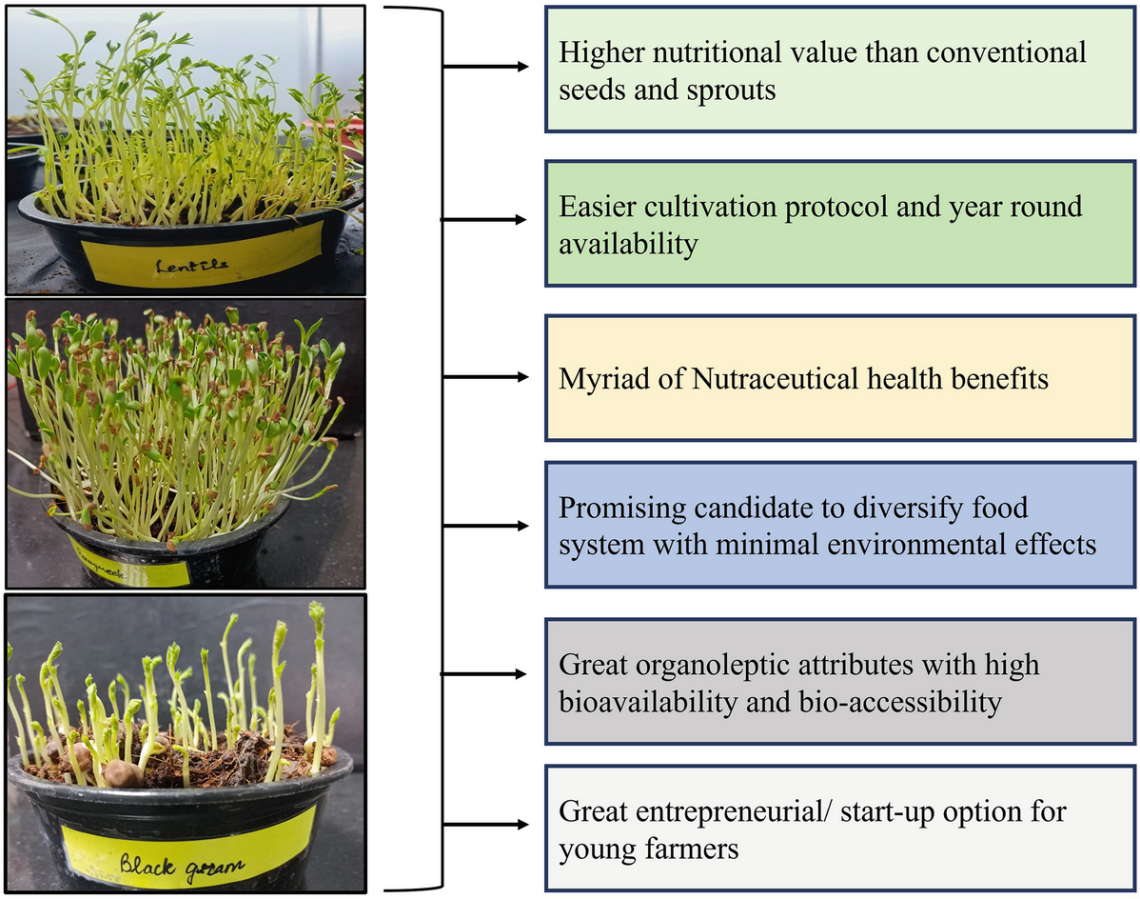


Fig. 1 The diagram representing the leguminous microgreens along with their multifarious sustainable and nutritional properties

3 Growing Parameters Considered for Leguminous Microgreens

Growing conditions like porosity and water holding capacity of media, duration of light exposure, humidity, temperature, seed density and irrigation are the key factors which are to be considered for the cultivation of microgreens. Some of them are discussed here. Growing media can play a crucial role for cultivation of leguminous microgreens and attain optimal physical, biochemical and nutritional attributes. Medium consisted of cocopeat, vermiculite and sand in ratio of 2:1:1

was provided for the growth of mung bean and lentil and the grown microgreens were harvested on day 7 and 9 respectively, after sowing the seeds when they reached the optimum stage (Priti et al. 2021). Growing media can play a crucial role for cultivation of leguminous microgreens and attain optimal physical, biochemical and nutritional attributes. Growing conditions like porosity and water holding capacity of media, duration of light exposure, humidity, temperature, seed density and irrigation are the key points which are to be considered for the cultivation of microgreens. The combination of cocopeat, vermiculite and sand in 2:1:1 ratio was found best growing medium pertaining to its higher water holding capacity. With this combination, the microgreens grown in plastic trays needed irrigation once a day or rarely twice for optimum growth (Di Gioia et al. 2015).

In a study, increased microgreen yield was reported with the increase in seed density. But when it reached to an optimum seed density, the growth and marketable quality of microgreens got depreciated. The microgreen yield at a seed density of 2, 3, and 4 seeds/cm^2 were studied and 3 seeds/cm^2 was found optimum for the growth of both mungbean and lentil microgreens (Priti et al. 2022). Further, high seed density hampers aeration and favours the fungal growth (Murphy and Pill 2010). A general growing protocol for the microgreens is represented in Fig. 2.

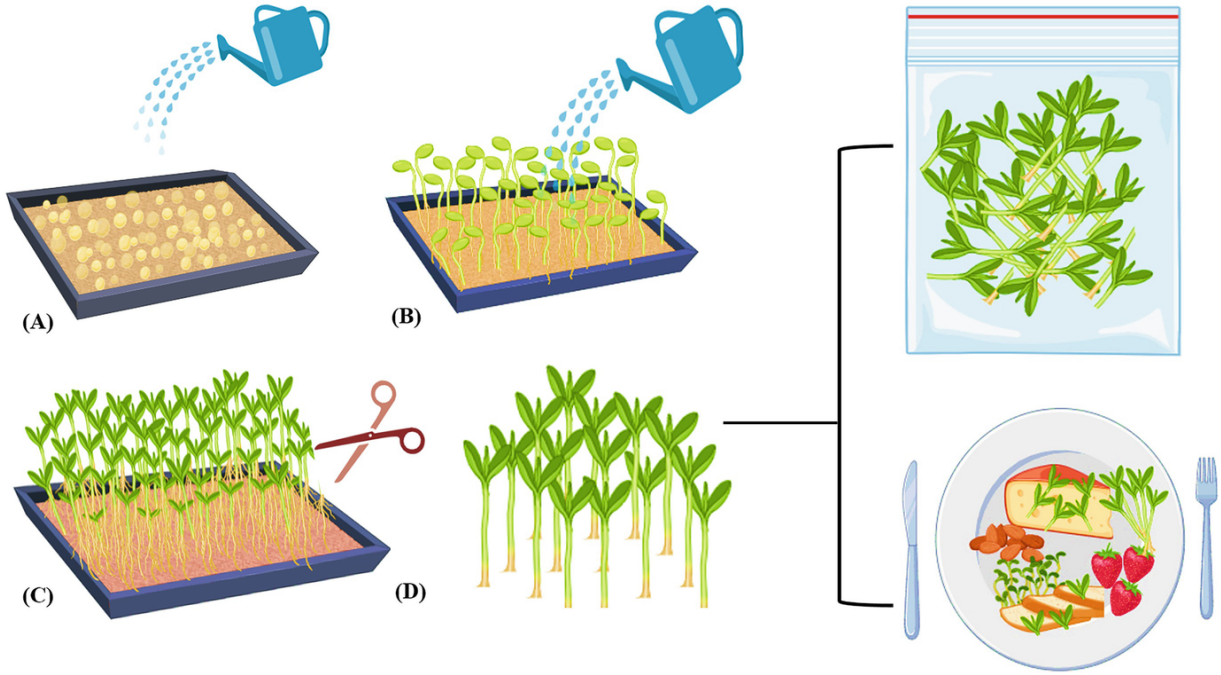


Fig. 2 A schematic representation of the cultivation process of leguminous microgreens (**a**) seed broadcast over a sterilized media, layering followed by water spray (**b**) Germination of seedlings within 3 days of sowing (**c**) Fully grown microgreens after 10 days with a pair of cotyledonous leaves, a delicate stem and roots (**d**) Harvested microgreens to be served as ready to eat superfood and for commercial or post-harvest benefits

Light plays a crucial role for the growth of plants and as well as production and accumulation of several phytocompounds. Mungbean microgreens cultivated under LED light showed increased growth as compare to fluorescent (room light). Early sprouting was also observed in LED light condition as the seeds started to germinate on day 2 whereas in case of room light it was seen on fourth day. However, the number of shoots were higher in room light condition as compared to that of LED light during first harvest (Manickavasagan 2021). Far-red light is reported to have inhibitory effect on fresh weight and hypocotyl length on soybean microgreens. The fresh weight of soybean microgreens was reduced by 16.30% when grown under far-red light as compared to that in white light condition. However, under other light spectra no significant difference in fresh weight and hypocotyl length was reported. Decreased yield of soybean microgreen was reported under far-red light

and UV-A. Further, total phenolic content and total flavonoid content were also found in higher concentrations in blue light and UV-A signifying the fact that blue light and UV-A are ideal monochromatic light which promotes the accumulation of phenolic compounds in microgreens (Zhang et al. 2019).

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4 Post Harvesting Handling

The post-harvest handling is one the major setbacks as far as the cultivation and commercialization of any genera of microgreens. However, the shelf life of leguminous microgreens can be extended by providing the low temperature storage conditions. As per a study, two storage packaging including the PPE transparent zip lock bags and aluminium foil containers were used to store the harvested microgreens at low temperature (Arya et al. 2023). The shelf lives of horse gram and green gram microgreens can be increased up to 9 and days respectively by keeping them at 4 °C in PPE zip lock bags. Besides, the storage at room temperature in zip lock bags would work for maximising the storage life up to 4 days only. The visual quality assessment of microgreens was taken into account for shelf-life studies based on a 5-point Hedonic scale as reported by Berba et al. (Berba and Uchanski 2012). The aluminium foil containers can be one of the choices for shorts term storage i.e. up to 2 days provided with the low temperature environment. Since the aluminium foil packages does not possess the proper air tight environment and hence is comparatively not worthy for post-harvest storage of microgreens. However, the air-tight packaging like PPE zip lock bags is always ideal to avoid moisture loss due to dehydration. The packaging film prevents the delicate textures from dehydration and from other external contaminants, thereby extending the shelf lives of microgreens (Turner et al. 2020). Both the visual and phytochemical properties of microgreens are highly dependent on their post-harvest storage conditions nonetheless, the freshly harvested

microgreens are always the best choice for exclusive functional benefits.

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5 Therapeutic and Health Effects of Leguminous Microgreens as Superfood

The nutritional deficiency is the major cause of various diseases and chronic ailments leading to a range of health disorders. The bioactive phytochemicals including polyphenols, flavonoids, minerals, vitamins and their precursors play a remarkable role in eliciting responses against multiple diseases thereby promoting the human health (Rizvi et al. 2023). Microgreens have recently evolved as newly introduced genera of superfood with the outstanding nutritional assets. It is served a great culinary component in fine dines owing to its spectacular organoleptic attributes like vibrant colours, delicate textures, elicited flavours and aroma (Caracciolo et al. 2020; Treadwell et al. 2020). An exceptionally rich nutrient properties and desirable sensory attributes have acclaimed the microgreens among consumers as an augmented superfood (Lenzi et al. 2019; Renna and Paradiso 2020). The higher concentration of vitamins, minerals and numerous bioactive compounds (like carotenoids, betaine, polyphenols, glucosinolates, saponins, poly-sterols, etc.) in microgreens have contributed for its incredible nutritional and functional aspects (Xiao et al. 2012; Kyriacou et al. 2019). As far as the leguminous microgreens are concerned, the genotypes of lentils and mung bean microgreens (L830 and MH810 respectively) were reported to possess substantially improved levels of total ascorbic acid, flavonoids, carotenoids, phenolic content and antioxidant activity (Priti et al. 2021). Owing to the great bioactive profiling, microgreens are well known to have anti-cancer, anti-diabetic, anti-inflammatory, anti-obese and other health promoting characteristics (Choe et al. 2018; Rizvi et al. 2023). Microgreens play crucial role in influencing the various immunological pathways which further persuade the other

metabolic consequences. The leguminous microgreens are densely packed with polyphenols which aid in disrupting the NF-κB pathway (nuclear factor light chain enhancer of activated B cell pathway). NF-κB protein complex is associated to cause inflammation by ultimately allowing the expression of pro-inflammatory cytokines like TNF-α, interleukin-1 (IL-1, IL-6 and IL-8) (Baldwin Jr 1996; Ruiz and Haller 2006; Gupta et al. 2010; Kanigur-Sultuybek et al. 2020). Additionally, polyphenols are also involved in inhibiting the NF-κB- DNA interaction (Khan et al. 2020). Moreover, the chlorophyll content was reported to be highest in green gram and horse gram microgreens ranging up to 14–16 mg/g when grown under the rain shelter with light intensity of about 117.82 μ mol m^{-2} s^{-1} at 29 °C (Arya et al. 2023). The polyamine densities are also found substantially improved in alfalfa, fenugreek and lentil microgreens. Alfalfa microgreens are merited for highest agmatine levels (5392 mg/kg) followed by cadaverine and putrescine (3563 and 1079 mg/kg respectively) in fenugreek microgreens. The spermine and spermidine levels were studied to be highest in fenugreek (922 mg/kg) and lentils microgreens (579 mg/kg) respectively (Cigić et al. 2020). Polyamines act as substrate for diamine oxidases which undergoes enzymatic oxidation to produce H_2O_2 which ultimately is involved in apoptosis and cell wall differentiation. This phenomenon is directly associated in antimicrobial activities during the tissue injury (Tavladoraki et al. 2016; Paschalidis et al. 2019). The germinating seedlings of legumes show the highest diamine oxidases expression levels. The hypocotyl and root systems of soybeans and chickpea are the predominant sites for the expressions of the enzyme (Scoccianti et al. 1990; Torrigiani and Scoccianti 1995). The oxidative deamination of bioactive amines induced by enzymatic actions may reduce the severity of migraine effectively (Izquierdo-Casas et al. 2019). Furthermore, the spermidine and spermine are known to be associated with cardioprotective and neuroprotective roles while agmatine can meticulously cross the blood brain barrier to relieve the CNS

associated disorders including the depression (Laube and Bernstein 2017; Madeo et al. 2018). The endogenous polyamine synthesis decreases with aging and therefore, the dietary intake of polyamines by means of leguminous microgreens can effectively reduce the mortality rates (Kiechl et al. 2018). The soybean microgreens were studied to have a significantly increased levels of phenolic and flavonoid content while grown under blue light and ultraviolet-A (UV-A) light modules. Over 66 bioactive phenolic metabolites (isoflavones, phenolic acid and flavonol) were identified to be consistent with the increasing antioxidant capacities (Zhang et al. 2019). Iso-flavonoids are one the most eminent bioactive phytochemical marker of Fabaceae family. Various perennial legumes have shown the biosynthesis of some major iso-flavonoids including genistein, daidzein, formononetin and biochanin A. Soybeans (Hedley 2000; Masood et al. 2014), red clover (*Trifolium pratense*), mung beans (*Vigna radiata*) (de Machado et al. 2009), fava bean (*Vicia faba*), and alfalfa (*Medicago sativa*) are some major dietary isoflavones (Devi et al. 2015). The highest concentration of formononetin and biochanin-A was found in zigzag clover (*Trifolium medium*) microgreens with 117.5 ± 6.3 and 33.4 ± 2.1 mg/100 g respectively. Additionally, the genistein and daidzein levels (2.7 ± 0.3 and 17.4 ± 1.4 mg/100 g respectively) were also reported to be comparatively higher in microgreens than that of sprouted counterparts. The therapeutic and nutraceutical properties exhibited by some of phyto-active compounds present in leguminous microgreens are discussed in Table 1.

Table 1 Therapeutic applications of bioactive phytocompounds of leguminous microgreen

Bioactive compounds	**Therapeutic applications**	**References**
Agmatine	Antidepressant effect in humans (2–3 g/day), Pain relief in humans related to radiculopathy (0.75–2 g/day)	Keynan et al. (2010) and Shopsin (2013)

Bioactive compounds	Therapeutic applications	References
Putrescine	Improves intestinal immune health in piglets and extend the lifespan in mice	Soda et al. (2009) and Liu et al. (2019)
Spermidine	Increased lifespan in mice, prevents aging and promotes liver regeneration in rats	LaRocca et al. (2013), Eisenberg et al. (2016), and Okumura et al. (2016)
Spermine	Extends the lifespan in mice, induces the antidiabetic effects, Showed increased antioxidant capacity in liver and spleen during oxidative stress, Induces liver regeneration in rats, Alleviate the anti-inflammatory and immune response in piglets	Sadasivan et al. (2014), Eisenberg et al. (2016), Okumura et al. (2016), Cao et al. (2017), and Wu et al. (2017)
Formononetin	Protects against high glucose-induced apoptosis in RSC96 Schwann cells, Improved the mechanical properties of the bones in rats with osteoporosis induced by ovariectomy on administration of formononetin for 4 weeks	Jiang et al. (2023)
Biochanin A	**Anti-cancer effect** Suppresses the ZEB1/PD-L1 axis to inhibit tumor growth, Activating the caspase-3 and Bcl-2 pathways, Checks the invasive enzymes expressions by regulating the multiple signaling pathways in Her-2 positive SK-BR-3 breast cell line. Activate the caspase 9 and 3 to inhibit the growth of osteosarcoma **Antioxidant effect** Defends the Hep-G2 cells against various cytoprotective enzymes such as NQO1 and	Nishimura et al. (2006), Al Bulushi et al. (2009), Kalač (2014), Wunderlichová et al. (2014), Laube and Bernstein (2017), Kiechl et al. (2018), Madeo et al. (2018), Butkute et al. (2019), del Rio et al. (2019), and Paschalidis et al. (2019)

Bioactive compounds	Therapeutic applications	References
	HO-1 induce by tert-butyl hydroperoxide, The decrease in SOD and CAT and increase in MDA, Nrf-2, NQO1 and HO-1 levels by activation of Nrf-2/ARE pathway, Reduces dopamine induced by lipopolysaccharide (LPS), inhibits overproduction of necrotizing factor, nitric oxide and superoxide produced by microglia **Anti-inflammatory effect** Reduces TNF-α, interleukins (IL-1β) production by inhibiting TLR4-MARK/NF-κB signaling. The increased Nrf-2 levels and NLRP3 inflammasome activation **Hypoglycemic effect** Enhanced SIRT1 expression in pancreatic tissues	
Daidzein	Anti-oxidative effects in muscles Improves the irritable bowel syndrome in females Also shows synergistic effects in association with vitamin D	Jalili et al. (2016)
Genistein	Helps in reducing weight gain as well as increase in bone mass in female obese mice, Reduces skin aging by improving the wrinkling and dryness of skin and thus used in cosmetic preparations	Polito et al. (2012), Michelin et al. (2016), and Simperova et al. (2016)

The flavonoids are already known to have free radical scavenging activities which remarkably activates the various cellular antioxidant mechanisms including the protection against low density lipoproteins (Katan 1997; Fuhrman and Aviram 2001; Warnakulasuriya et al. 2014; Sharifi-Rad et al.

2022). Furthermore, the flavonoids can significantly regulate the prostaglandin production by inhibiting cyclooxygenase-2 (COX-2) levels which ultimately regulates the NF-κB pathway to induce the anti-inflammatory responses (Subbaramaiah et al. 1998; Wang et al. 2020; Ahsana et al. 2021).

The overall proximate profiling of perennial leguminous microgreens comprised a substantial amount of protein (44.0–58.7 g/100 g), carbohydrates (20.0–29.7 g/100 g) and fat (4.13–6.48 g/100 g) contents (Table 2). Among other legumes, *O. viciifolia* microgreens was studied to possess the highest protein concentrations of about 58.7 g/100 g which was comparatively the highest as possessed by other counterparts of the same crop. The seeds and sprouts contained 35.8 g/100 g and 45.2 g/100 g of protein content respectively (Butkute et al. 2019). Microgreens of seeded perennial legumes (*M. sativa, M. lupulina, O. viciifolia*) were also studied to have higher concentrations of secondary metabolites viz. coumesterol, triterpenes saponins and tannins. The concentrations of isoflavones were found abundantly in clover species of microgreens (141 mg/100 g) as compared to the seeds and sprout counterparts of the same species (10.8 and 60.9 mg/100 g respectively). As far as the elemental profiling is concerned, the potassium content was reported highest among all macro-elements in all the counterparts. Interestingly, the microgreens of all legume species displayed the average of 1.59 g/100 g while the seeds and sprouts possessed 1.35 and 1.37 g/100 g of potassium (Butkute et al. 2019). Macro and micro elements present in leguminous microgreens are discussed in Tables 3 and 4. Besides, the microelements (zinc and iron) are present enormously in both the sprouted seeds and microgreens of perennial legumes thereby prospecting them for food fortification. The global micronutrient deficiency particularly of zinc and iron, which is often called as ‘hidden hunger’, can be greatly reduced by augmenting the leguminous sprouts and microgreens in the main stream of human diet specially children below 5 years and women (Gregory et al. 2017; La Frano et al. 2014). In fact,

the iron and zinc deficiency in vegetarian diet is one of the current hypes as far as the nutritional security is concerned (Hunt 2003). The other leguminous microgreens like green gram were reported to have rich elemental profiles involving the calcium, potassium and iron up to 29.93%, 27.49%, and 1.28% respectively (Ghoora et al. 2020). Such meticulous role of bioactive phenolic components can greatly help in reducing the chronic ailments like cardiovascular and inflammatory. Therefore, the leguminous microgreens are highly recommended to eradicate both micro and macronutrient deficiencies globally.

Table 2 Comparative proximate analysis of sprouts and microgreens of perennial leguminous crops

Perennial legumes	Forms of legumes	Crude fat (g/100 g)	Crude protein (g/100 g)	Total fiber (g/100 g)	Total carbohydrates (g/100 g)
Red clover	Sprouts	9.28 ± 0.01	38.9 ± 0.09	15.6 ± 0.88	31.8
	Microgreens	4.84 ± 0.23	45.7 ± 0.43	17.5 ± 1.06	26.1
Alfalfa	Sprouts	8.88 ± 0.33	42.8 ± 0.38	14.0 ± 0.45	29.7
	Microgreens	6.48 ± 0.21	44.0 ± 0.93	14.5 ± 0.32	29.7
Black medick	Sprouts	5.14 ± 0.24	42.6 ± 0.13	13.9 ± 0.67	34.0
	Microgreens	4.13 ± 0.24	48.0 ± 0.14	14.4 ± 0.69	27.4
Sainfoin	Sprouts	5.58 ± 0.03	45.2 ± 0.41	11.4 ± 0.54	32.5
	Microgreens	5.26 ± 0.16	58.7 ± 1.04	8.7 ± 0.47	20.0

Table 3 Micro-elements reported in mungbean, lentil and perennial legumes

Micro-elements (mg/100 g of fresh weight)				
Microgreens	**Fe**	**Zn**	**Cu**	**Mn**
Mungbean	0.59 ± 0.011 (MH521)	0.26 ± 0.009 (TM96-2)	0.10 ± 0.0009 (IPM02-3)	0.19 ± 0.013 (PMR-1)
Lentil	0.75 ± 0.032 (L4602)	0.40 ± 0.025 (IPL321)	0.18 ± 0.007 (L4076)	0.15 ± 0.004 (KLS218)
Perennial legume microgreens	**Fe**	**Zn**	**Cu**	**Mn**
Sainfoin	19.8 ± 0.511	13.3 ± 1.24	–	–

Micro-elements (mg/100 g of fresh weight)				
Alfalfa	17.8 ± 0.902	27.3 ± 1.69	–	–
Red clover	16.5 ± 0.434	29.9 ± 1.50	–	–
Cicer milkvetch	21.5 ± 0.69	30.0 ± 1.57	–	–
Black medick	14.8 ± 1.13	17.9 ± 0.921	–	–

Table 4 Macro-elements reported in mungbean, lentil and perennial legumes

Macro-elements					
Microgreens	**Ca**	**K**	**Mg**	**P**	**Na**
	(in mg/100 g)				
Mungbean	86 ± 2.6 (TM96-2)	3695 ± 14.9 (MH96-1)	56 ± 2.6 (IPM409-4)	87 ± 2 (Pusa Ratna)	60 ± 5.57 (IPM02-3)
Lentil	70 ± 5.29 (JL-1)	389 ± 12.77 (L4076)	66 ± 4.58 (KLS218)	38 ± 3 (L4602)	45 ± 3.61 (DPL62)
Perennial legume microgreens	**Ca**	**K**	**Mg**	**P**	**Na**
	(in g/100 g)				
Sainfoin	0.585 ± 0.038	1.98 ± 0.01	0.299 ± 0.001	1.001 ± 0.009	–
Alfalfa	0.611 ± 0.019	1.49 ± 0.01	0.373 ± 0.068	0.878 ± 0.006	–
Red clover	0.238 ± 0.012	1.34 ± 0.06	0.358 ± 0.016	0.819 ± 0.008	–
Cicer milkvetch	0.339 ± 0.038	1.81 ± 0.03	0.326 ± 0.022	–	–
Black medick	0.702 ± 0.024	1.13 ± 0.04	0.293 ± 0.004	0.852 ± 0.001	

6 Commercial and Future Prospects of Leguminous Microgreens

Microgreens are grown on a large scale to provide a functionally important high value food for human consumption due to their vast nutritional-rich profiling. The commercial cultivation of such superfood can be a potentially viable idea for young entrepreneurs and farmers, especially after COVID pandemic. From the past recent years, microgreen consumption has been chosen to enrich the mainstream diets with vital bioactive components without even going to

supermarkets. The appealing textures and overall sensory attributes of microgreens has drawn the attention of specific consumer preferences. As per the findings by uninformed consumer's evaluation, the aesthetic attributes of microgreens were the major choices including the flavours and textures for their approval. The lesser astringency, sourness and bitterness in flavours was the general criterion for approval. However, such sensory and phytochemical based evolutionary merits are necessary to explore the understanding behind the bioactivity thereby, extrapolating their hype to market launch. Besides, the developing countries like India, should follow health awareness regimes to publicize the science behind nutritional foods like microgreens. The shorter growth lifecycle, simpler media requirements, sustainability and remarkable nutritional densities are certain key features of microgreens allowing us to cultivate and commercialize in Indian markets to earn a handsome profit. As far the entrepreneurial strategy is concerned, one can smartly opt for B2C (business to consumer) to specific consumer segments viz. elderly population, pregnant females, diet planners, gym freaks, etc. The commercialization of microgreens can be extrapolated to B2B (business to business) for example, five-star hotels, fine dine restaurants, cafes and eateries (Fig. 3). Moreover, the digital marketing platforms and e-commerce can be explored as well to sell the ready to use kits and fully grown packs of microgreens among various customers. It can be a great measure to draw the consumer's interest in augmenting the microgreens as dietary supplements by directly growing their own superfood in homestead or opting the commercial marketing for the same. To summarize, the cultivation of microgreens is comparatively much easier to initiate from our homes itself as a small start-up for young entrepreneurs, farmers and fitness enthusiasts to cope with economical as well as malnutrition lacunae in developing countries. The personnel involved in production of microgreens may further visit food processing units and packaging industries to improve their skills and explore the market demanding

agricultural as well as highly nutritional products. Following some essential growing and marketing tactics, microgreens can be placed among foremost nutritionally rich foods and therapeutics.



Fig. 3 Prospective clients for commercializing microgreens on various platforms

7 Major Challenges and Limitations in Microgreen Cultivation

With the increasing concept of health and functional foods, a global surge in microgreen farming has taken the rise in food marketing and industry. Due to lesser popularity among the common health foods, microgreens specifically the leguminous ones display less dispensable consumer market which is a major challenge for commercializing the microgreens. The

hype and glare of microgreens as a recently introduced superfood in launch has led to a higher market price. On the other hand, the pathogenic contamination is another major challenge during the overall growth phases, harvest and final packaging of microgreens (Olaimat and Holley 2012; Alegbeleye et al. 2018). The study revealed the high-risk prominence during the cultivation stages due to microbial and viral exposures from growth practices and animal, human or other plant physiological interventions (Riggio et al. 2019). Sometimes the biochemical constituents from crop surface plays key role in increased yield susceptibility to contamination for example, the strains of *Salmonella and E. coli* can bind to the molecules of lectins and (Bennett et al. 2018; Berger et al. 2010; Gao et al. 2016; Olaimat and Holley 2012; Wang et al. 2017). Microgreens are often handpicked during harvesting times resulting in causing contamination due to improper sanitization. However, the contamination challenges can be taken care of by following the adequate hygiene protocols and sanitization technologies (Rizvi et al. 2023). Another setback with microgreen farming is the lack of awareness among the common masses of society. Despite being nutritionally dense functional food, popularising the consumption of microgreens among all sections of masses is yet another challenging task to accomplish. Furthermore, the post-harvest handling, shorter lifespan, quick senescence and comparatively lower yields are some of the critical issues related to the production and commercialization which should be addressed (Chandra et al. 2012; Kou et al. 2013; Puccinelli et al. 2019).

8 Conclusion

Microgreens are new class of superfood highly rich in nutrients and biochemical profiles. These are known to form an indispensable portion of human diet to combat various ailments and deficiencies. Apart from a variety of microgreens grown worldwide, the leguminous microgreens are one of the

least studied yet the nutritionally promising microgreens thus promote human health remarkably. The several studies have done till date revealed the fact that leguminous microgreens contain higher amounts of bioactive phytochemicals including polyamines, polyphenols like flavonols, isoflavones, etc. The other secondary metabolites viz. coumesterol, triterpenes, saponins and tannins are also reported to be present enormously in leguminous microgreens. The antioxidant capacities of such microgreens are substantially higher as compared to their counterparts (seeds, sprouts and mature plants). Owing to rich bioactive compositions, leguminous microgreens may play crucial role in preventing various ailments like inflammations, cardiovascular issues and even cancers. Furthermore, they are exclusively rich in both macronutrients and micronutrients thereby bridging the gap of hunger and nutritional security globally. Despite the various challenges, microgreens as therapeutic food can be augmented as strategic supplement along with other vegetables and legumes to ensure a complete balanced diet especially in the developing nations like India. Moreover, the young entrepreneurs and farmers of the nation can sustainably opt for microgreens' farming and commercialize them over digital and e-commerce platforms to ensure better profits in a shorter time.

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Nutritional Composition of Leguminous Microgreens

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Abbreviations

Ca Calcium

CEA Control Environmental Agriculture

Fe Iron

HDL High-density lipoprotein

K Potassium

LDL Low-density lipoprotein

Mg Magnesium

Zn Zinc

1 Introduction

Microgreens were introduced to the public on a San Francisco chef's menu in the late 1980s. When Craig Hartman first used the term "microgreens" in 1992, his friend Michael Clark was growing and supplying them. Microgreens gained attraction in California, USA, during the mid-1990s. While the exact definition of microgreens remains somewhat ambiguous, numerous publications characterize them as a distinct type of vegetable cultivated from vegetable or herb seeds. They bear a resemblance to sprouts but are cultivated for a longer duration and, unlike sprouts, are typically grown under light conditions. Microgreens also Known by another name, "vegetable confetti," microgreens were originally added as garnishes to improve salads, soups, sandwiches, and some dishes (Treadwell et al. 2020; Kyriacou et al. 2016). Microgreens, come from variety of sources viz; cereals, vegetables, and herbs, are a step between infant leaf vegetables and sprouts. They are made up of three parts: a stem, cotyledon leaves/the first pair of genuine leaves. They are harvested between 7 and 21 days of germination measuring about 2.0–8.0 cm in height (Galieni et al. 2020). Microgreens act as a boon to this era as the growing interest of society is mainly focused on eating healthy and fresh food (Ebert 2022). However, they add a distinct flavor, attractive color, pleasant aroma, and smooth texture.

Because of their high concentration of bioactive ingredients, including phytochemicals (phenolics, anthocyanins, betacyanins, and carotenoids), vitamins (A, C, E, K) and minerals (copper, zinc, selenium, manganese, potassium, sulfur and molybdenum), microgreens are referred to as the next generation of "superfoods" or "functional foods." contains nine times as many nutrients are present as in mature plants (Xiao et al. 2012). Microgreens also have minimal anti-nutritional factors, unique growth patterns that need less area, low care costs, and a short growing season. With all of these qualities,

microgreens are considered an excellent treatment for conditions including cancer, obesity, heart problems, neurological diseases, malnutrition, etc. (Xiao et al. 2012; Zhanga et al. 2021).

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2 Difference Between Sprouts and Microgreens

In recent years consumption of microscale green vegetables has taken its own pace (Galieni et al. 2020). Sprouts are a category of plants whose seeds can be consumed whereas shoots and leaves of microgreens are edible to consume. However, consumption of sprouts causes health issues. Both sprouts and microgreens have higher nutritional content than seeds or fully-grown plants. Additionally, because big macromolecules are broken down by enzymes, microgreens and sprouts are rich in simple sugars, fatty acids, and amino acids as well as bioactive substances and phytochemicals (Benincasa et al. 2019; Mir et al. 2017; Renna et al. 2018, 2020; Ghoora et al. 2020).

Consuming sprouts has been linked to several food-borne illnesses. However, no such cases have happened that are associated with microgreens (Galieni et al. 2020). Sprouts and microgreens differ in various ways: Sprouts are consumed in their raw stage with their roots. But microgreens undergo a seed-soaking period, after which they are allowed to grow on substrate. When these micro plants grow up to 5 cm, the roots have been removed and consumed after cooking. As a result, microgreens are considered as considerably safer than sprouts, which are regarded as “high-risk” items. Nutritious food with few low-risk categories viz; fresh herbs including basil, thyme, etc. and leafy vegetables (baby spinach) (Ding et al. 2013; Warriner and Smal 2014; Riggio et al. 2019; NSW Food

Authority. Products Food Safety Scheme Periodic Review of the Risk; 2020).

Sprouts are a good source of antioxidants like polyphenol and L-ascorbic acid. Compared to microgreens, they are richer in sugars, pectins, and amino acids. However, compared to sprouts, microgreens had a higher concentration of carotenoids, chlorophyll, and organic acid. Microgreens contain no added sugar which is considered as the major advantage behind their consumption. They also showed stronger anti-diabetic and anti-cholinergic properties. As functional foods or superfoods, a few chosen sprouts (broccoli, radish, lentil) and microgreens (radish, amaranths, kale) should be consumed every day (Wojdyło et al. 2020). Sprouts and microgreens both have high pH levels exceeding 5.3 and titratable acidities of less than 0.7 and 0.4 g/100 fw, respectively. The amounts of oxalic acid in microgreens are ten times higher than those in sprouts (Wojdyło et al. 2020). Further, Microgreens are packed with antioxidants such as glucosinolates, carotenoids, and phenolic compounds, along with essential vitamins like C, E, and K, minerals such as iron and magnesium, and other vital nutrients including fiber and omega-3 fatty acids. Research suggests that incorporating microgreens into one’s diet can contribute to cardiovascular health, inflammation reduction, cancer prevention, diabetes management, nervous system protection, and enhanced digestion (Tallei et al. 2024).

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3 Physical Characteristics of Microgreens

Microgreens are known to be a functional food with unique properties such as color, flavor, etc., and bioactive substances enhance their health-promoting properties and hence increase their demand (Janovská et al. 2010;

Sirtautas et al. 2012). This chapter provides a comprehensive understanding of the various factors influencing the physiology of microgreens, from pre-sowing treatments to post-harvest handling. Primarily during pre-sowing treatments, it is necessary to explore various treatments applied before sowing that positively impact the germination physiology of microgreen seeds. Secondly, examines the influence of light on microgreens growth processes, the quantity and quality of light, and its effect on the agglomeration of bioactive substances in microgreens (Brazaityté et al. 2013, 2015; Kou et al. 2014). Further in the post-harvest stage, it is essential to avoid the presence of microorganisms in harvested microgreens which prolongs the shelf-life of microgreens and upholds their quality even after harvest. Microgreens, or young vegetable greens, are harvested soon after the cotyledons' leaves emerge (the first leaves that appear during germination) have developed. They are typically harvested when they are very young and small, usually around 1.5–3 inches in height. These tiny plants are known for their tender, delicate texture, color, and intense flavor, often resembling the taste of the mature plant but concentrated in a smaller package. A few microgreens with their physical parameters such as length, color, and flavor are listed in the Table.1.

Table 1 Physical characteristics of various microgreens

Microgreens	Length (Inches)	Color	flavor	References
Lettuce, Tropaeolum, Tom Thumb, Parsley	2–3	Light green.	Mild	Xiao et al. (2015), Bhatt and Sharma (2018), Stoleru et al. (2016), and Hendriks (2021).
Radish red	2.5–3	Red/purple	Spicy	
Red cabbage	1–3	Purplish green	Mild and peppery	
Magenta spinach	3	Red	Slightly sweet	
Soyabean	2–3	Green	Neutral & Distinctive	
Chickpea	4 cm	Deep black seeds with vibrant green shoots	A sweet pea-like flavor with a citrus zing	
Lentil	2–3	Whitish to light purple	Mild earthy	
Alfalfa	0.5–2	Deep green	Mild nutty	
Pea shoot	4–6 inches (10–15 cm)	Vibrant green	Sweet, tender taste and earthy flavor	
Basil	1–2	Aromatic green	Sweet to cinnamon–like	
Cauliflower	2	Dark green	Mild and fresh broccoli flavor	
Mustard	3–4	Green	Spicy	
Broccoli	2–3	Green	Bitter	
Buckwheat	2	Leafy green	Sour or tangy	

4 Cultivation of Microgreens

Microgreens are highly versatile and can be cultivated at varying scales, ranging from small-scale, home-based cultivation to large-scale industrial production for commercial purposes. The scale of cultivation doesn't only influence the quantity produced but can also affect the cultivation, harvesting, and postharvest handling practices (Mehta and Kumar 2022). In the current scenario, microgreens are gaining more attractiveness due to their high nutritive contents. However, the growth of microgreens is comparatively easier than traditional crops with fewer resources in a short period, and do not require any pesticides or herbicides. The global movement toward Control Environmental Agriculture (CEA) is driven by concerns about sustainability, a decrease in disease or pests in agriculture, and adequate food (Turner et al. 2020).

Protected cropping, indoor farming; vertical farming are some of the methods of microgreen cultivation in a controlled environment. Microgreens encompass a wide variety of crops and crop varieties, including those from the Brassicaceae family, as well as vegetables, grains, and legumes. These are similar to sprouts but take more time for germinate under specific lighting conditions. They can be cultivated throughout the year and harvested between 7 and 14 days after germination containing two cotyledons and a pair of first true leaves (Enssle 2020). The initial investment for microgreens production is relatively low. They are widely used for dishes like salads, soups, sandwiches, etc., as they increase flavor and color (Treadwell et al. 2010; Xiao et al. 2012). The detailed glimpse into the practices and characteristics of microgreen cultivation, shedding light on irrigation methods, varieties grown, growing systems, and also the source of information for growers showcases the diversity and adaptability of this emerging agricultural sector. Sustainable ways of watering techniques like drip irrigation

(33%), overhead watering (23.9%), etc. are adopted to grow these microgreens (Misra and Gibson 2021). These statistics suggest a variety of irrigation practices among microgreen growers, with a preference for bottom watering/drip irrigation.

Growers cultivated a diverse range of microgreen varieties, including Radish, Sunflower, Pea shoots, Arugula, Broccoli, Kale, Mustard, Basil, Pea Tendrils, Cabbage, Mizuna, Beet, Amaranth, Cilantro, Nasturtium, Kohlrabi, and Popcorn. Respondents used different growing systems, including climate-controlled greenhouses (6 growers), indoor commercial spaces (3 growers), and indoor residential spaces (24 growers). Peat moss (17.6%), organic soil (15.3%), and coco coir (14.2%) are the mediums used for the cultivation of microgreens. Additives such as perlite and vermiculite were also mentioned. The growing medium represents one of the most critical aspects with a considerable impact on the productivity, quality, and safety of microgreens (Nidasesi and Malladadavar 2020). Scientists made several attempts to substitute soil substrates like peat and polyethylene terephthalate for the growth of microgreens. However, these soil substrates are costly and not able to renew with other cost-effective substrates like textile fiber and jute-kenaf fiber (Di Gioia et al. 2017). The growth of microgreens depends on the quality of seeds with good germination ability of around 95% and also the seeds must be free of microorganisms and other contaminants. Another important factor influencing the growth and formation of phytochemicals in microgreens is the source of light. Few microgreens like Amaranth, Arugula, Cilantro, etc. are grown well in the presence of incidental sun rays but several studies have proven that man-made sources of light such as Light Emitting Diode (LED) which produces visible radiation are effective in microgreen production (Bula et al. 1991).

Tap water (32.4%), well water (29.5%), rainwater (2.8%), and surface water (1.1%) are the major water sources involved in the irrigation of microgreen farmlands. The Microgreen production industry requires more exposure for their development which can be achieved through websites and online videos. Informal learning from other growers constituted 12.5% of the learning sources. Microgreens differ from sprouts in terms of seed-soaking period and harvesting. Microgreens have a shorter seed-soaking period and are harvested after the cotyledons have developed and true leaves are emerging. Microgreens are consumed without the roots (Galieni et al. 2020).

Integrated multiomics approaches, combined with nutriomics and foodomics, offer promising avenues for identifying and breeding the most potent microgreen genotypes. These approaches can be utilized to enhance the nutritional content, including macro-elements such as potassium (K), calcium (Ca), and magnesium (Mg), as well as oligo-elements like iron (Fe) and zinc (Zn), along with boosting antioxidant activity. Furthermore, integrated approaches can target other desirable traits in microgreens, such as fast growth, optimal nutritional values, high germination rates, and extended shelf-life. The implementation of integrated approaches involves various cutting-edge technologies, including genomics, transcriptomics, sequencing-based methodologies, molecular breeding techniques, machine learning algorithms, nanoparticle applications, and seed priming strategies. By leveraging these advanced tools and strategies, researchers can unlock the full potential of microgreens, enhancing their nutritional quality and overall performance for improved health benefits and sustainable food production (Gupta et al. 2023).

4.1 Optimum Conditions for the Growth of Microgreens

Microgreens are generally more successful in warmer temperatures, and the range you mentioned (20–30 °C) is suitable for their rapid germination and growth. Different microgreens may have specific temperature preferences (Dhaka et al. 2023). Some varieties may tolerate slightly cooler or warmer temperatures. Cooler temperatures might slow down the germination and growth of microgreens. Using a heat mat under the trays can provide bottom heat and help maintain a warmer soil temperature, especially in colder environments. While warmer temperatures can promote faster growth, it's crucial to avoid excessive heat that may lead to issues like drying out the growing medium or causing heat stress to the plants. Adequate ventilation is essential to prevent heat buildup, especially in indoor environments. Sudden temperature fluctuations can stress the microgreens, affecting their growth and development. Hence regular monitoring of temperature and humidity is very much necessary. Maintaining the right humidity levels is essential for the successful germination and growth of microgreens. The range of around 70–80% humidity, is generally suitable for most microgreens (Hernández-Adasme et al. 2023). During the germination stage, it's often beneficial to provide higher humidity to create a moist environment that encourages seed germination. As the microgreens grow, it's essential to gradually reduce humidity to prevent issues such as mold and fungal diseases. Specific microgreens may have slightly different humidity preferences, so it is always necessary to check the recommendations for the particular varieties of growth of microgreens. Additionally, the ambient conditions in a growing environment, such as temperature and air circulation, can also influence humidity levels, so it's essential to consider these factors collectively for successful microgreen cultivation. The temperature and humidity levels play a vital role in every stage of plant growth i.e. from germination to harvesting. These optimum

conditions favor good nutrient content in microgreens along with wholesome growth, desired flavor, and attractive color.

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5 Classification of Microgreens

Microgreens can be categorized according to several factors, including taste, color, and botanical family. Tasting microgreens is one method of classification. Certain microgreens, including microgreens of spinach and lettuce, have a moderate flavor. These greens are well-liked for their delicate flavor and adaptability in cooking. However, certain varieties of microgreens have a powerful flavor that gives meals a boost. Examples are the peppery flavor of radish and mustard microgreens or the distinct herbaceous flavor of cilantro microgreens.

Color is an additional categorization criterion. Microgreens are available in a variety of colors, ranging from vivid reds and purples to deep green. Amaranth microgreens are striking tones of scarlet, and red cabbage microgreens have a lovely purple. These greens' vibrant range of colors not only makes food seem better but also signals the presence of many health-promoting phytonutrients.

Also, the botanical family of microgreens can be used to categorize them effectively. Asteraceae (the daisy family), Apiaceae (the carrot family), Brassicaceae (the mustard family), and Amaranthaceae (the amaranth family) are examples of common families. Every family possesses distinct attributes about flavor, consistency, and nutritional composition. List of popular microgreens has been shown in Table 2.

Table 2 Classes of popular Micro greens families with percentage use

Family	Examples	Percentage usage
Amaranthaceae	*Beta vulgaris L. Alternathera sessilis, A. tricolour L, Amaranthus viridis, Amaranthus caudatus L. Amaranthus graecizans L, Amaranthus oleraceous L. Spinacea oleracea, A. spinosus L*	95%
Poaceae	*Zea mays, Eleusine coracana, Setaria italic, Sorghum vulgare, Cymbopogon citrates, Panicum sumatrense, Avena sativa, Pennisetum glaucum, Triticum aestivum, Fagopyrum tataricum L.*	
Amaryllidaceae	*Allium cepa*	2.44
Pedaliaceae	*Sesamum indicum,*	
Apiaceae	*Pimpinella anisum L., Daucus carota, Apium graveolens L., Coriandrum sativum, Foeniculum vulgare Mill, Apium graveolens L.*	12.19%
Asteraceae	*Tagetes spp, Helianthus annus,*	4.87
Brassicaceae	*Brassica oleracea L. var capitata L, Brassica oleracea L. var botrytis L., Brassica juncea, Raphanus sativus, Brassica rapa,* *Eruca sativa Mill,* *Lepidium bonariense L*	9.75%.
Linaceae	*Linum usitatissimum L.*	2.44
Cucurbitaceae	*Cucumis sativus L., Cucurbita maxima*	4.87%
Lamiaceae	*Ocimum sanctum L, Mentha arvensis, Ocimum basilicum L*	
Fabaceae	*Cicer arietium L, Trigonella foenicum-graecum L., Vigna radiata, Pisum sativum, Lupinus angustifolius*	9.75%

Source: Parida (2020) and Kumar et al. (2018)

5.1 Leguminosae Microgreens

Fabaceae or Leguminosae is the second largest family of flowering plants. Leguminosae is also termed as the pea family. Plants are called legumes. Legumes are considered

to be rich in many bioactive components and phytochemicals (Altuner 2021). Some leguminous microgreens include chickpeas, clover, lentils, alfalfa, pea, fenugreek, sunflower, flax, etc. Flavonoids and carotenoids present in leguminous microgreens make them a potent antioxidant. These antioxidants help protect cells from damage caused by harmful free radicals.

5.1.1 Trigonella foenumgraecum

This legume is commonly called methi having clover-shaped leaves. Fenugreek microgreens are aromatic with a bitter flavor and are added to salads, curries, and dishes.

5.1.1.1 Nutritional Benefits

This microgreen has a low calorific value and is nutrient-dense, enriched with vitamins such as A, C, and E, and minerals like Fe, Ca, and K. It is also rich in dietary fiber and antioxidants (Singh et al. 2020).

5.1.1.2 Health Benefits

(a) Vitamin C helps boost the immune system and collagen production and acts as an antioxidant. The dietary fiber mainly aids in a healthy gut.

(b) The antioxidants like flavonoids and phenols protect the cells from oxidative stress which can lead to early aging and diseases.

(c) Fenugreek microgreens act as a rich source of protein diet especially for vegetarians and vegans. These proteins are essential for the growth, repair, and maintenance of cells.

(d) Folic acid maintains the production of red blood corpuscles and DNA production.

(e) Regular consumption of fenugreek microgreens regulates blood sugar levels in diabetic and prediabetic patients.

(f) Phytoestrogen level of Fenugreek microgreens helps in decreasing menstrual discomforts (Wadhawan et al. 2017; Bhaswant et al. 2023).

5.1.2 Lupinus angustifolius

Lupin Microgreens, with their subtle flavor profile, are often incorporated into bread making. Research findings revealed that “lupin microgreen bread” contained a substantial 87% of the total flavonoid content, whereas the “lupin leaf bread” contained 22%.

5.1.2.1 Nutritional Benefits

(a) Excellent flavonoid source.

(b) Lupin microgreens are superior to lupin leaves in providing isoflavone, particularly genistein, a potent antioxidant.

(c) Non-processed lupin microgreens contain 15 chrysoeriol glycosides, along with lutein, zeaxanthin, and β-carotene (Klopsch et al. 2018).

5.1.3 Vigna radiata

Commonly known as green gram belongs to the family Fabaceae mainly found in south-east Asia. The microgreen has long netted leaves with a mild nutty taste. These microgreens are tiny and tender and harvested within a week. They serve as an excellent ingredient in salads, soups, sandwiches, and fries.

5.1.3.1 Nutritional Benefits

For over a millennium, it has served as a traditional remedy in China, boasting a wealth of proteins, minerals, and antioxidants (Kaur et al. 2022). It stands out as an abundant source of potassium (K), phosphorus (P), and calcium (Ca) (Priti et al. 2021).

5.1.3.2 Health Benefits

These microgreens are the best source of plant-based protein with compact essential amino acids like leucine, lysine, isoleucine, phenylalanine, etc. with low calorific value.

(a) Green gram microgreen contains antioxidants like flavonoids, phenols, cinnamic acid, etc. These antioxidants neutralize the free radicals and protect the body from inflammation, heart diseases, and various types of cancer like lung cancer and stomach cancer.

(b) Vitexin and isovitexin are the two important antioxidants present in this microgreen which can fight against heat strokes resulting from the formation of free radicals.

(c) The antioxidants present can also lower the LDL cholesterol preventing free radical interaction.

(d) They contain minerals like magnesium and potassium which help in the regulation of high blood pressure in patients.

(e) Butyrate present in green gram microgreen helps to maintain healthy colon cells and prevent colon cancer.

(f) Ghrelin is the hormone responsible for hunger, the fibers and proteins help in the suppression of this hormone which in turn leads to weight loss.

(g) Green gram microgreen is rich in folic acid, fiber, and iron which serve as an excellent source of these nutrients in pregnant ladies and promote healthy growth of the fetus.

5.1.4 Phaseolus vulgaris

Phaseolus Vulgaris comes under the family of common beans. It is an herbaceous annual plant that grows everywhere. This leguminous plant consists of edible dry seeds and green, unripe pods. Its leaf is used as a vegetable and the straw as fodder. It comes under the legume family Fabaceae.

5.1.4.1 Nutritional Benefits

These microgreens are vital sources of polyphenolic substances having wide health benefits. They are rich in dietary fiber, minerals, vitamins, carbohydrates, and proteins (Hayat et al. 2014). It comprises abundant quantities of flavonoids like anthocyanins, proanthocyanidins, flavonols, etc. along with phytochemicals and antioxidants (Beninger and Hosfi 2003; Choung et al. 2003).

5.1.4.2 Health Benefits

(a) In our daily consumption, microgreens provide both physiological and health benefits against heart-related disorders, obesity, diabetes, cancer, etc. (Jenkins 2007; Chung et al. 2008; Hayat et al. 2014)

(b) It exhibits good antioxidant and anticarcinogenic properties due to its rich functional and nutraceutical constituents which exist as phytochemicals (Cardador-Martínez et al. 2002; Dinelli et al. 2006).

(c) It plays a vital role in controlling the growth of free radical species due to the presence of phenolic

substances that inhibit enzymatic activity or bind with the metallic ions to form a complex during the process of radical formation and hence has very good antimutagenic and antioxidative properties (Decker 1995; Elizabeth et al. 2007).

(d) Another advantageous aspect of incorporating beans into one's diet is their ability to reduce total cholesterol and LDL cholesterol levels, making them particularly beneficial for individuals experiencing symptoms of metabolic syndrome (Anderson et al. 2002). Anderson et al. in 1990 did a clinical trial to prove the health benefits of beans. According to their study, consumption of these beans for 3 weeks by hypercholesterolemic patients resulted in the reduction of serum cholesterol and LDL cholesterol by 19–24%. This dietary fiber lowers the cholesterol level as it helps to excrete total steroids and also prevents neutral sterols and bile acids absorption by the intestine (Moundras et al. 1997).

(e) Phaseolus Vulgaris mainly reduces the risk of heart and its related disorders as proved by many clinical studies on cardiac-related diseases (Finley et al. 2007; Winham and Hutchins 2007; Hayat et al. 2014).

5.1.5 Pisum sativum

Pisum Sativum now renamed as Lathyrus oleraceus, is a group of peas belonging to the Fabaceae plant family well known as pulse. Many seeds will be there in a single pod that seems to be green or yellow, as it gets matured. Peas are annual plants, with a life cycle of 1 year.

5.1.5.1 Nutritional Benefits

Peas contain minerals including magnesium, potassium, and calcium which are essential for the cardiovascular

system. In addition, peas are very helpful in building the immune system as they comprise antioxidants like zinc, catechin, vitamin E, etc. Carotenoids, flavonols, and vitamin C are protective and regulate the proper functioning of cardiac vessels.

5.1.5.2 Health Benefits

(a) The nutrient carotenoids such as lutein and zeaxanthin present in these peas protect our eyes from chronic diseases and also damage caused due to aging. The harmful blue light causing macular degeneration and cataracts is removed by a membrane i.e. Lutein and Zeaxanthin carotenoids.

(b) These are vital and fight against cancer cells in the stomach due to the coumestrol constituent of peas. A previous survey done in the year 2009, in Mexico revealed that 50% risk caused by cancer cells was reduced by increasing the intake of these peas daily.

(c) The digestion and excretion process is made easy by the consumption of these peas due to their high dietary fiber content.

(d) These peas are abundant with antioxidants like vitamin C, vitamin E, Zinc, Catechin, and Epicatechin that strengthen the immunity of our body.

(e) As these peas are enriched with protein and dietary fibers which control the body’s metabolism and reduce blood sugar levels. A recent case study proved that a regular high-protein diet reduces sugar levels in type 2 diabetic patients. Also, sudden rises in sugar levels can be regulated by consuming peapods having a low glycemic index.

(f) Reduces heart risks: These peas facilitate the reduction of swelling or infection leading to the generation of plaque on the walls of the blood vessels caused by oxidation of free radicals. The content of fatty acids in peas like Omega-3 and Omega-6 reduces infection and thus prevents plaque generation.

5.1.6 Trifolium pratense

This bean is a herbaceous species belonging to the Fabaceae family originating from Europe, Western Asia, and Northwest Africa but cultivated throughout the world.

5.1.6.1 Nutritional Benefits

Red clover's primary component is isoflavones, which are found in various plants and mimic the function of estrogens. Additionally, red clover contains a range of nutrients such as calcium, chromium, magnesium, niacin, phosphorus, potassium, thiamine, and vitamin C. Notably, red clover exhibits significant antioxidant properties, boasting the highest total antioxidant activity at 4789.373 mg TE g^{-1}, along with a substantial phenolic content of 791.770 mg GAE 100^{-1} g^{-1} (Altuner et al. 2022).

5.1.6.2 Health Benefits

(a) It prevents the blood from clotting as red clover acts as a blood thinner. It appears to improve blood flow. Studies reveal the fact that red clover might help protect the body against heart disease. However human studies have no profound evidence. Red clover isoflavones have been associated with an increase in HDL ("good") cholesterol in pre and postmenopausal women, but other studies show conflicting results. One study found that menopausal women taking red clover supplements had stronger, more flexible

arteries (called arterial compliance), which can help prevent heart disease.

(b) Red clover shows promise in alleviating symptoms like hot flashes and night sweats due to its estrogen-like properties. However, current research findings are inconclusive. While some studies on a specific extract of red clover isoflavones indicate a notable reduction in hot flashes among menopausal women, the results from the largest study did not demonstrate any significant effect.

(c) During menopause, due to estrogen levels drop, a woman's risk for developing osteoporosis (significant bone loss) goes up. Few types of research suggest that extract of red clover isoflavones may have an impact on bone loss and even boost bone mineral density in pre-and perimenopausal women. However, there is scope for more research in this area.

(d) Researchers have initiated investigations into the potential of isoflavones derived from red clover for cancer prevention and treatment, building upon its traditional usage. Early research findings indicate that these isoflavones could inhibit the growth or induce the death of cancer cells in laboratory tests. Red clover might offer protection against certain types of cancer, including prostate and endometrial cancer. However, due to its estrogenic properties, it could also promote the growth of certain cancers, similar to estrogen.

(e) Traditionally, red clover ointments have been applied to the skin to treat psoriasis, eczema, and other rashes. Red clover has also been used as a cough remedy for children. More recently, studies have

shown that women using red clover may experience psychological benefits.

5.1.7 *Vicia faba*

Vicia faba, commonly known as the broad bean, fava bean, or fava bean, is a species of vetch, a flowering plant in the pea and bean family Fabaceae.

5.1.7.1 Nutritional Benefits

Nutritionally, mature seeds of fava beans are rich in proteins (26.1%), carbohydrates (58.3%), and dietary fiber (25.0%) (USDA 2021). Fava beans also contain various bioactive compounds like total phenolics and flavonoids, which act as antioxidants (Valente et al. 2018). However, fava beans also contain antinutritional factors such as lectins, saponins, trypsin inhibitors, phytic acid, condensed tannins, and favism-inducing factors, which negatively affect their biological value (Revilla 2015; Dhull et al. 2022).

5.1.7.2 Health Benefits

Fava Beans help to manage Parkinson's which is associated with weakened motor performance. Fava beans increase the levels of L-dopa, a chemical that improves motor performance (Broad bean (*Vicia faba*) consumption and Parkinson's disease. Rabey et al. 1993).

5.1.8 *Glycine max*

This legume is commonly called Soybean and belongs to the Fabaceae family which has green leaves. It has a low calorie of 85 calories suitable for a weight-loss diet. Soy bean microgreens have a neutral distinctive flavour and are added to salads, curries, and dishes.

5.1.8.1 Nutritional Benefits

These are the source of essential nutrients like vitamin K, vitamin C, and vitamin B. It is enriched with protein, dietary fiber, and minerals such as iron, potassium, and manganese.

5.1.8.2 Health Benefits

(a) Vitamin B and folate are essential for the synthesis of DNA and red blood cells.

(b) Prevents high blood pressure.

(c) Prevents and reduces the growth of cancer cells.

(d) Antioxidants prevent the risk of developing age-related macular degeneration (AMD) in the eyes.

5.1.9 Cicer arientum

This legume commonly known as Chickpea (also known as *Garbanzo* or Chana) belongs to the family of Fabaceae and has dark black seeds and vibrant green shoots which have the flavor of sweet pea with a citrus zing. They are low in calories and can be used as a healthy snack or side dish.

5.1.9.1 Nutritional Benefits

These microgreens are rich in minerals like iron and calcium. It contains Vitamin B1, B2, B3, B5, and B6. Cooked chickpea contains 46.9% carb, 6.3% fats, 20.9% other nutrients, and fiber content of 13.6%.

5.1.10 Health Benefits

These are rich in plant-based protein especially for vegetarians and provide good muscle health.

(a) Dietary fiber: Helps easy digestion and assists in weight management.

(b) Minerals and Vitamins: Overall well-being

(c) Antioxidants: This content prevents oxidative stress and reduces the risk of chronic disorders.

5.1.11 Lens culinaris

This belongs to the Fabaceae family and is well known as Lentil. It has a whitish to light purplish color with a mild earthy flavor and peppery taste. It is used in various dishes as one of the constituents or as toppings. It is fat-free with fewer calories.

5.1.11.1 Nutritional Benefits

Lentils are renowned as a nutritional powerhouse owing to their rich content of iron, magnesium, vitamin B, and vitamin C. Additionally, they exhibit high levels of chlorophyll, with the highest total chlorophyll (36.632 µg g^{-1} TA), chlorophyll a (25.247 µg g^{-1} TA), chlorophyll b (11.385 µg g^{-1} TA), and carotenoids (7.015 µg g^{-1} TA) (Altuner et al. 2022). Lentils also provide significant amounts of potassium (K), calcium (Ca), and phosphorus (P). Furthermore, they are recognized for their total phenolics content, total flavonoid content, and antioxidant activity (Priti et al. 2021).

5.1.11.2 Health Benefits

(a) As it is rich in iron it benefits menstruating women.

(b) Stimulates strength in muscles.

(c) Reduces the threat of atherosclerosis.

(d) Pull down cholesterol levels.

(e) Declines menace of ailments like Alzheimer's, cataracts, osteoarthritis, and diabetes.

(f) Supports optimal brain functioning.

(g) Aids in soothing vascular muscles in the heart.

(h) Support in weight management.

(i) Decreases risk of cancer.

5.1.12 Medicago sativa

This belongs to the Fabaceae family and is commonly known as Alfalfa or Lucerne. It is one of the most nutritious microgreens available. It has a mild nutty flavor with dark green. It is widely known as the "Father of all foods". It has extremely low calories of 23 calories.

5.1.12.1 Nutritional Benefits

It is a rich source of antioxidants and has nutrients like vitamin K, magnesium, copper, and folate.

5.1.12.2 Health Benefits

(a) Reduces cholesterol level.

(b) Helps in blood sugar management.

(c) Relieving symptoms of menopause.

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6 Conclusion

Microgreens, tender, edible seedlings vary in their taste but are rich in vitamin contents. Microgreens are known as 'superfoods' because of their rich bioactive ingredients. In

addition, they contain phytochemicals and minerals that are used for cancer treatment, obesity, heart problems, neurological diseases, malnutrition, etc. Sprouts are also legumes whose seeds can be consumed but shoots and leaves are edible parts of microgreens. Sprouts consumption can lead to several food-borne illnesses. However, no such cases have happened which are associated with microgreens.

Microgreens need special pre-sowing treatments, have the effect of sunlight on quality, quantity, and agglomeration of bioactive substances in microgreens, and need avoidance of microorganisms post-harvest. Although microgreens are cultivated in wide conditions (domestic, commercial, small scale, and large scale), their cultivation is well endorsed in controlled environments. During cultivation we need to choose the right soil or soil substrates, the right seed variety, irrigation by drip irrigation or overhead irrigation, and exposure to the right quantity of sunlight. Microgreens can flourish in different ranges of temperature based on the type of plants. However, germination happens mostly in warmer temperatures and care should be taken to avoid high variation of temperature and ample ventilation and humidity. They are divided into various groups according to their taste, color, and botanical family. Legume microgreens are high in antioxidant phytochemicals like carotenoids and minerals like copper and zinc. They are also high in vitamins like ascorbic acid. Legume microgreen on the other hand is much tastier, gluten-free, and rich in antioxidants, carbohydrates, proteins, minerals, and fibres. Greengram varieties are rich in proteins, minerals, and antioxidants and have benefits for controlling cholesterol and colon cancer. Fabaceae is the largest flowering plant. Legumes in Fabaceae are plants rich in many bioactive components, phytochemicals and antioxidants which serve as shields against free radicals

that cause cell damage. Trigonella foenumgraecum microgreen or methi has a low calorific value but is rich in vitamins, dietary fiber, minerals, and calcium. Fenugreek microgreens act as a rich source of protein diet especially for vegetarians and vegans. These proteins are essential for the growth, repair, and maintenance of cells. Buckwheat microgreens have a mild nutty flavor used in smoothies, salads, and sandwiches. Buckwheat microgreens are gluten-free and rich in antioxidants, carbohydrates, proteins, minerals, and fibers. Green gram was used as an ancient medicine in China which is rich in proteins, minerals, and antioxidants.

Phaseolus Vulgaris L provides nutrients, minerals, and vitamins. It also known to protect against some serious illnesses, including cancer, diabetes, and cardiovascular disease. *Pisum Sativum*, coming under pulse is a rich source of minerals including magnesium, potassium and calcium and antioxidants help to build the immune system, Eye health, Digestive health, Blood sugar control, etc. *Trifolium pratense*, a herbaceous species of flowering plant, has blood-thinning properties, helps to reduce symptoms of menopause such as hot flashes and night sweats, has an impact on bone loss, and has anti-cancer activity. The broad bean, also called *Vicia faba*, raises L-dopa, which enhances motor function.

Microgreens of *Glycine Max*, *Cicer Arientinum*, *Lentil*, *Medicago sativa,* etc. have immense health benefits including reduction of atherosclerosis, Support in weight management, reduce cancer, Support in optimum brain functioning, etc. The above information reveals the fact that microgreens and sprouts are factories of natural proteins and nutrients.

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Microgreens: A Novel Source of Functional Nutraceuticals

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Abbreviation

NCD Non Communicable Diseases

1 Introduction

Over the past 10 years, there has been a global increase in people's desire to consume fresh, healthful, and functional foods like microgreens and sprouted seeds, particularly during and after the COVID-19 epidemic. Beyond basic nutrition, a wide variety of foods or their ingredients can be classified as functional foods or natural health benefit

products due to their diversity of bioactive components that successfully promote health and prevent diseases (Butkutė et al. 2018). Legume seeds and their sprouted products, which include high levels of protein, carbs, minerals, and other health-promoting phytonutrients, are known for their multifaceted qualities. Consumer interest in microgreens has increased, particularly among high-end restaurant chefs who utilize a variety of microgreens as garnishes to improve salads, soups, sandwiches, and other culinary enhancements (Kyriacou et al. 2016; Lone and Pandey 2024). Vegans who are cautious about consuming foods use microgreens as a source of raw foods. They are young, immature plants that are harvested when the first true leaves appear (Riggio et al. 2019). Legume, vegetable, or plant microgreens are high in nutrients, minerals, and phytochemicals. Microgreens replaced sprouts due to their high nutritional value, strong flavor, and taste. Compared to their mature counterparts, microgreens have significantly higher quantities of vitamins and carotenoids —roughly five times on average. Increased heart health, and decreased risk of osteoporosis, Alzheimer's disease, and cancer are all facilitated by such high nutritional levels. They are rich in vitamins like C, E, and K, as well as essential minerals. Several pre- and post-harvest practices, including light exposure, salt stress, nutrient enhancement, and natural media substrates can improve the nutritional content of microgreens (Dereje et al. 2023). Microgreens have a high antioxidant capacity (Tallei et al. 2024) and are useful in regulating the metabolism of cholesterol and plasma lipoproteins because of their richness in vitamins and phytochemicals. This suggests that microgreens may be useful in the prevention and/or treatment of chronic disorders. Additionally, they often contain higher levels of beneficial phytonutrients compared to fully matured plants. Including microgreens in a balanced diet can contribute to overall health and well-being. They're thus referred to as

functional or superfoods. Varieties of microgreens include cauliflower, broccoli, cabbage, radish, carrot, garlic onion, Amaranthus, beetroot, spinach, melon cucumber, cereals such as rice, oats, wheat, corn, and barley, as well as legumes like chickpeas, beans, and lentils. Microgreens are a delicious and nutritious addition to your diet. Each type boasts a unique flavor profile, ranging from mild and sweet to peppery and spicy. They are an enticing option for health-conscious people and food enthusiasts alike for their concentrated nutritional content, vivid flavors, and ease of home growing (Butkutė et al. 2018).

Leguminous microgreens are a subset of microgreens that belong to the legume family, Fabaceae. These microgreens are derived from the seeds of leguminous plants and are known for their unique flavors, nutritional benefits, and the ability to fix nitrogen in the soil which can be beneficial for overall soil health and sustainability in agriculture. Whether used as a garnish, in salads, or as a main ingredient, leguminous microgreens contribute to the growing trend of incorporating fresh and nutrient-dense greens into everyday meals. Legumes like mung beans, lentils, and chickpeas pack a surprising nutritional punch when sprouted into microgreens. They boast concentrated levels of vitamins and minerals like iron, zinc, and vitamin C, often exceeding their mature counterparts (Bhaswant et al. 2023). This makes them a valuable addition for those seeking a nutrient boost, especially vegans and vegetarians. Mung beans offer a sweet, grassy note, while lentil microgreens have a slightly earthy, peppery bite. Chickpea microgreens bring a nutty richness to the table. These varied taste profiles add complexity and excitement to salads, sandwiches, dips, and even smoothies. Studies suggest that leguminous microgreens may offer some health benefits, including boosting immunity, aiding digestion, and reducing inflammation. While more research is needed, their impressive nutrient profile suggests they

can be a valuable part of a healthy diet. Leguminous microgreens are surprisingly easy to grow at home, even without a garden. With minimal space and light, we can harvest this tiny nutritive source within a week (Zhang et al. 2020). This makes them a sustainable and eco-friendly option compared to commercially grown greens.

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2 Microgreen-Phytochemical Constituents

Leguminous microgreens boast impressive levels of antioxidant compounds like flavonoids, phenolic acids, and carotenoids (García-Tenesaca et al. 2024). These potent phytochemicals scavenge free radicals, protecting cells from damage and potentially reducing the risk of chronic diseases like cancer and heart disease. Some studies suggest that specific isoflavones and saponins in leguminous microgreens possess anti-inflammatory properties. This could be beneficial for individuals suffering from conditions like arthritis or inflammatory bowel disease. Certain prebiotics and dietary fiber found in leguminous microgreens can promote the growth of beneficial gut bacteria. This improved gut health is linked to better digestion, stronger immunity, and even mood regulation. These tiny plants are often rich in essential minerals like iron, zinc, and magnesium. This makes them particularly valuable for individuals with dietary deficiencies or those at risk of anemia. The phytochemical profile of leguminous microgreens varies depending on the specific legume and growing conditions. This diversity offers a range of potential health benefits and keeps nutritional analysis exciting.

Microgreens are superior to sprouts in terms of their phytochemical composition including phenolics and carotenoids, and sensory qualities. Due to their high

nutrient content, microgreens in lesser quantities will have nutritional benefits comparable to those of greater quantities of mature veggies. Young vegetable greens are known as microgreens. Both sprouts and microgreens are known to have lower antinutritional factors than seeds or fully-grown plants but have higher levels of amino acids, fatty acids, and simple sugars. Nutrient-dense sprouts and microgreens may be grown quickly since they have a short development cycle. Microgreen's dry matter content is approximately 5% of their fresh mass, while sprouted seeds have several times more moisture content than seeds. The amounts of lutein, neoxanthin, α-carotene, β-carotene, and violaxanthin were higher in amaranth microgreens than in sprouts (Ebert et al. 2015). *Brassica* species microgreens was found to contain. Polyphenols such as 30 anthocyanin, 105 flavanol glycosides, and 29 hydroxycinnamic acids (Sun et al. 2013). Microgreens have more complex polyphenol profiles and higher contents than mature *Brassica* plants, making them good sources of antioxidants (Zhang et al. 2021).

On a comparative study of the seeds, sprouts, and microgreens of clover species with *Medicago*, *Onobrychis* and *Astragalus* species, the former accumulated several times more isoflavones than the others. In *T. medium* seeds, two isoflavones (formononetin and biochanin A) were quantified while only traces of these isoflavones, detected in seeds of other plant species such as *Trifolium pratense*, *Medicago sativa*, *M. lupulina*, *Onobrychis viciifolia*, *Astragalus glycyphyllos* and *A. cicer* species studied. All of the examined legumes raw seeds had daidzein and genistein quantities below the quantitative limit. All bean species germinated products contained quantifiable amounts of formononetin and biochanin A, with microgreens containing the greatest concentrations. In chickpea seeds, the rise in formononetin and biochanin was

the primary cause for the 100-fold increase in total isoflavone contents. The microgreens were generally the source of the highest total concentration of isoflavones, with the raw and sprouted seeds coming in second and third. The concentrations of formononetin in *M. lupulina* and *O. viciifolia*, as well as biochanin A in *M. sativa*, *M. lupulina*, *O. viciifolia*, and *A. cicer*, did not alter significantly between sprouted seeds and microgreens. The extracts of all the samples of *M. sativa*, *M. lupulina*, *O. viciifolia*, and *A. glycyphyllos*, *A. cicer* had only trace amounts of genistein and daidzein. The contents of non-phenolic metabolites such as triterpene saponins and other phenolic compounds like condensed tannins and coumestrol increased in the same order as isoflavone concentration. The greater being microgreens followed by sprouted seeds and less in seeds. *M. sativa* seeds and microgreens had considerably more triterpene sapnins than *M. lupulina*, and *M. lupulina* samples had a much higher content of coumestrol than the corresponding *M. sativa* samples. The amount of saponin in the sprouted seeds of *M. sativa* and *M. lupulina* did not significantly differ (Zhang et al. 2021).

Microgreens and leaves from pea and lupin plants are good, natural components to boost health-promoting secondary plant metabolites. More flavonoids, carotenoids, and chlorophylls were found in the leaves of lupin (*Lupinus angustifolius*) and pea (*Pisum sativum*). Pea was primarily composed of acylated quercetin and kaempferol sophorotrioses. In lupin, genistein and malonylated chrysoeriol were the main components. Both of the pea tissues had high concentrations of quercetin glycosides, indicating that quercetin can be found in both the leaves and microgreens of the plant. Chrysoeriol accumulation was greater in lupin leaves. Thus, dietary recommendations for lupins might be customized to each person’s needs,

depending on the anticarcinogenic properties of genistein-rich microgreens or the antidiabetic effects of chrysoeriol-rich leaves (Klopsch et al. 2018).

The Brassicaceae microgreens included zinc, selenium, lutein, beta carotene, anthocyanins, polyphenols, and zeaxanthin. While selenium content was highest in little burnet and common dandelion, zinc contents were greater in wild mustard. In comparison to other species, pak choi and tatsoi were discovered to have a higher total chlorophyll content. The amount of lutein in mustard was low, but jute had higher levels (Kyriacou et al. 2019). Kohlrabi also had lower levels of β-carotene. Wild mustard contains the highest concentration of total anthocyanins when compared to common dandelion and small burnet (Lenzi et al. 2019). Mustard had a far greater anthocyanin content than watercress, broccoli, daikon, and rocket salad. However, it was found that broccoli had the highest content of total polyphenols while mustard had the lowest (Marchioni et al. 2021). Microgreens had a good amount of β-carotene, but their concentration of lutein/zeaxanthin was higher than other microgreens in cilantro, red sorrel, red cabbage, and garnet amaranth (Xiao et al. 2016). Variances in the bioactive compounds of microgreens may be primarily caused by differences in their genotypes, which include variances in their photosynthetic and metabolic activities. The higher amount of Vitamin A, Vitamin E, and carotenoids present in microgreens contributed to their overall nutritional quality.

Legumes in all the stages of growth showed a considerable amount of protein which was higher in microgreens (44.0–58.7 g/100 g) followed by sprouts seeds (937.0–45.2 g/100 g) and seeds (32.0–36.1 g/100 g). The crude fat content was found to be 2.85–11.6 g/100 g in seeds, 3.95–9.28 g/100 g in sprouts, and 4.13–6.48 g/100 g in microgreens. The ash and carbohydrate contents were also high in microgreens (5.34–7.31 and 20.0–

29.7 g/100 g), and moderate levels in seeds and sprouts (3.97–8.19 and 36.1–46.0 g/100 g, 4.24–5.35 and 29.7–37.6 g/100 g) respectively. *O. viciifolia* at different phases of growth, *O. viciifolia* had the lowest concentration of crude fibre (CF) (12.2, 11.4, and 8.7 g/100 g) and the highest concentration of protein (35.8 g/100 g) in seeds, 45.2 g/100 g of sprouted seeds, and 58.7 g/100 g of microgreens. The samples of *M. lupulina* and *M. sativa* showed higher fat levels. Less protein (32.0 g/100 g and 37.0 g/100 g, respectively) and fat (2.85 g/100 g and 3.95 g/100 g, respectively) were found in the seeds and sprouted seeds of *A. glycyphyllos*, but more crude fat (16.2 g/100 g and 17.2 g/100 g, respectively) (Zhang et al. 2021).

In microgreens, sprouting seeds, and seeds (1.77, 1.67, and 1.98 g/100 g, respectively), *O. viciifolia* accumulated noticeably more potassium. In *Trifolium pratense* microgreens, Ca varied from 0.238 g/100 g. The *M. lupulina* samples had the considerably lowest Fe concentrations (9.20 mg/100 g in seeds, 13.9 mg/100 g in sprouted seeds, and 14.8 mg/100 g in microgreens) among the investigated species in samples of all ontogenic stages. Zinc concentrations were higher—over 6 mg/100 g—in the seeds of *Trifolium pratense*, *O. viciifolia*, and *A. cicer* compared to *M. lupulina* seeds, which had nearly twice as much zinc—3.35 mg/100 g. Perennial legume microgreens and sprouted seeds have higher zinc contents, which makes them viable sources for adding the element to the diet. It is, therefore, possible to speculate that the microgreens and sprouted seeds of perennial legumes are a rich source of Zn and Fe, and that their bioaccessibility may even be enhanced (Zhang et al. 2021).

The microgreens of cucumber (*Cucumis sativus* L.) and jute (*Corchoris olitorisu* L.) had more ascorbic acid than their mature stages. The water spinach's vitamin C

concentration was comparable in its mature and microgreen stages. In contrast to the microgreen stage, the mature plants of several species, such as Amaranthus (*Amaranthus tricolour* L.), bottle gourd (*Lagenaria siceraria* Standl), palak (*Beta vulgaris L. var. bengalensis* Roxb), pumpik (*Cucurbita moschata* Duchesne), and radish (*Raphanus raphanistrum*), displayed higher vitamin C (Yadav et al. 2018). Microgreen stages of fenugreek (*Trigonella foenum-graecum* L.), spinach (*Spinacia oleraceae* L. var.), and roselle (*Hibiscus sabdariffa* L.) had vitamin C contents that were 120%, 127%, and 119% higher than those of their mature stages. Ten commercially available microgreens were found to have ascorbic acid concentrations ranging from 29.9 to 123.2 mg/100 g fresh weight (Ghoora et al. 2020), which is similar to citrus fruits (Rastakhiz et al. 2015), a popular food source of ascorbic acid. In 25 commercially available microgreens, a range of total vitamin C contents (20.4–147.0 mg/100 g fresh weight) asserted that many of them had greater total vitamin C concentrations than their mature plants (Xiao et al. 2012). Since microgreens are often eaten raw, most of their flavor can be maintained without cooking.

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3 Role of Growth Condition on Nutritional Content of Microgreen

The nutritional content of microgreens is influenced by various factors, and growth conditions play a crucial role in determining their nutritional profile (Gunjal et al. 2024). Light intensity and spectral quality affect the growth and nutritional value of radish microgreens (Partap et al. 2023). This study found that higher light intensity increased vitamin C and B2 levels in radish microgreens, while blue light enhanced chlorophyll content. Different wavelengths of light can trigger the production of various

phytochemicals in microgreens. For example, blue light promotes the growth of stems and leaves, while red light encourages the synthesis of carotenoids, like beta-carotene, a precursor to vitamin A. Study on the Influence of different light spectra on the growth and bioactive compounds of pea shoots (Pisum sativum L.). Wang et al. showed that red and blue LED lights promoted higher antioxidant activity and total phenolic content in pea shoots compared to white light. Cooler temperatures (18 °C) led to higher vitamin C and K levels in broccoli microgreens compared to warmer temperatures (24 °C) (Partap et al. 2023). Rocket microgreens grown at lower temperatures (15 °C) had higher glucosinolate content, a group of bioactive compounds with potential health benefits (Demir et al. 2023). The usage of fertilizers enriched with specific minerals like iron and zinc increased their levels in the corresponding microgreens (Xiao et al. 2019). Hydroponically grown kale microgreens had higher levels of certain vitamins and minerals compared to soil-based microgreens, but lower levels of others (Puccinelli et al. 2019). The pH of the growing medium can affect nutrient availability to the plants. Maintaining an optimal pH level is essential for nutrient uptake and utilization. The growth conditions of microgreens, including light, temperature, water, nutrient density in the growing medium, harvest time, seed quality, cultivation practices, and pH levels, collectively contribute to the nutritional content of these young plants. Fine-tuning these factors based on the specific requirements of each microgreen variety can help maximize their nutritional value.

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4 Microgreens—Role of the Secondary Bioactive in Mitigating

Inflammation by Modulating the Immune System

The bioactive compounds in microgreens can help to reduce inflammation in several ways. For example, sulforaphane has been shown to inhibit the production of inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-alpha). Isothiocyanates have been shown to reduce the activity of NF-κB, a transcription factor that plays a key role in inflammation. In addition to their anti-inflammatory effects, microgreens also have several other health benefits. They are a good source of essential nutrients, such as vitamin C, vitamin K, and folate. They are also a good source of fiber, which can help to improve gut health (Liu et al. 2018; Wu et al. 2023a, b).

Inflammation is a complex physiological process triggered by the immune system in response to injury, infection, or stress. While acute inflammation is essential for healing and defense, chronic inflammation contributes to various diseases like cancer, heart disease, and autoimmune disorders. Microgreens, with their concentrated levels of bioactive compounds, have emerged as potential dietary modulators of inflammation (Nair 2023). These bioactive components, synthesized by the plant as secondary metabolites, are not directly involved in primary growth but offer various health benefits, including anti-inflammatory properties. Glucosinolates are sulfur-containing compounds found in Brassicaceae family microgreens (broccoli, kale, raddish) that break down into isothiocyanates upon tissue disruption, exhibiting potent anti-inflammatory and antioxidant effects. Studies have shown isothiocyanates to suppress pro-inflammatory cytokines and enzymes, thereby reducing inflammation. Carotenoids are pigments like beta-carotene and lutein present in leafy microgreens (kale, and spinach) that possess antioxidant and anti-inflammatory properties.

Carotenoids scavenge free radicals, preventing oxidative stress and inflammation. Additionally, they modulate immune cell function, favoring anti-inflammatory pathways (Baranska et al. 2012). Phenolic acids are diverse plant metabolites abundant in various microgreens (radish, beet, arugula) that exhibit anti-inflammatory properties by inhibiting pro-inflammatory enzymes and signaling pathways. Phenolic acids also scavenge free radicals and modulate immune cell function, contributing to the overall anti-inflammatory effect. These secondary bioactives present in microgreens interact with the immune system at various levels to mitigate inflammation. These bioactive compounds suppress the production of pro-inflammatory cytokines like IL-6 and TNF-α, promoting a shift towards an anti-inflammatory response. Certain bioactives like isothiocyanates and phenolic acids target and inhibit enzymes involved in inflammatory pathways, thereby reducing inflammation. Carotenoids and some phenolic acids activate signaling pathways that promote anti-inflammatory responses, further mitigating inflammation. Bioactive compounds can influence the activity and function of immune cells, favoring anti-inflammatory phenotypes and reducing pro-inflammatory activity (Tsao and Wu 2007) (Table 1).

Table 1 Bioactive and nutritional components in microgreens

Family	Common name	Bioactive components	Nutritional components
Brassicaceae	Broccoli, cabbage, cauliflower and radish	Sulforaphane, Carotenoid, Isothiocyanates polyphenols, anthocyanin, monoterpene hydrocarbons, phytol and ascorbic acid, phylloquinone, and tocopherols, flavonol, glycosides, hydroxycinnamic acid, hydroxybenzoic acid,	Vitamins (E, A, K), minerals (N, K, Ca, Fe) and chlorophyll contents

Family	Common name	Bioactive components	Nutritional components
		ascorbic acids and total sugars,	
Fabaceae	Green gram	Anthocyanins	Carbohydrates, proteins, fat, fiber, pantothenic acid (B5) and pyridoxine (B6)
	Chickpea	Anthocyanins, ascorbic acids	
	Fenugreek	Phenolic compounds, α-tocopherol phenolic and flavonoids	Potassium, and minerals (K, Ca, Na, Na, Cu, Fe and Cu)
Apiaceae	Carrot	Anthocyanins and carotenoids	–
Amaranthaceae	Beet	Total phenol, flavonoids, anthocyanins and α-tocopherol	Vitamins
Lamiaceae	Basil	Total ascorbic acid, phylloquinone, phenols, flavonoids, carotenoids, chicoric acid, α-tocopherol, anthocyanins, lutein, and anthocyanins	Potassium and magnesium

Partap et al. (2023)

5 Microgreens—Modulator of Lifestyle Disorders and Gut Microbiome

Obesity is a predisposing factor in the progression of both impaired glucose tolerance and diabetes mellitus. Obesity and uncontrolled Diabetes Mellitus (DM) are the root causes of the development of cardiovascular diseases (CVD) which are linked with hyperlipidemia, insulin resistance, and hyperinsulinemia. Prabhakaran and co-workers demonstrated that the mortality associated with

CVD cases has been estimated to be 54.5 million in India according to the Global Burden of Disease Study that was carried out from 1990 to 2016 (Prabhakaran et al. 2018). Prospective case and cohort studies exhibited that calorie-rich food, processed food, dairy food, and animal-based food, as well as a sedentary lifestyle, elevate the risk of obesity and associated disorders.

Dietary choices play a decisive role in managing clinical manifestations associated with metabolic complications. A poor diet can alter the gut microbiota leading to increased firmicutes to Bacteroides ratio thereby decreasing the production of short-chain fatty acid (SCFA), Glucagon like peptide-1, Peptide tyrosine tyrosine, serotonin, cholecystokinin and so on., which leads to adipogenesis, decreased insulin sensitivity, declined to scavenge potential that causes inflammation and oxidative stress that may lead to metabolic, behavioral, and neurological disorders.

Various observational and prospective studies have displayed that the inclusion of plant-based diets provides a long-term reversal of obesity, type-II DM, and induced CVDs (Jakše et al. 2022; Flores-Balderas et al. 2023). Aune and co-workers carried out a whole-food plant-based (WFPB) lifestyle program, for 2555 subjects aged 18–80 years. They provided three servings of whole grains per day for 10 years and observed a significant reduction in body mass index and body fat percentage in obese subjects (Aune et al. 2013). Similarly, various reports have suggested that the adequate consumption of fruits, vegetables, legumes, and nuts improves glucose metabolism, reduces cholesterol levels, and substantially inhibits the risk of developing obesity and type II DM. Dietary patterns accentuate the plant-based diets for mitigating type II DM-associated co-morbidities because of their integral properties, including high fiber content and anti-inflammatory, antioxidant, and immune-boosting metabolites (Salas-Salvadó et al. 2019).

Microgreens are enriched with protein (16–30%), amino acids, vitamins, minerals, antioxidants, and secondary metabolites, although only a handful of *in vivo* studies has been carried out, their effectuality in maintaining blood glucose levels (Durairajan et al. 2024) and managing weight as well as a significant reduction in white adipose tissue mass evidenced by *in vitro* and *in vivo* studies provide an insight on the modulatory capabilities of these minuscule plants in improvising gut health by attenuating alterations in carbohydrate, protein and lipid metabolism.

The following section will discuss the role of microgreens as the natural modulators of gut microbiota by elevating and alleviating various biochemical, molecular, and cellular factors as evidenced by various *in vitro* and *in vivo* studies. Wu et al. reported that consumption of red cabbage downregulated the AF12 gene expression which is positively correlated with weight gain and fecal bile acid in mice administered with a high-fat diet. However, an elevation in Firmicutes/Bacteroidetes (F/B) ratio was observed in the mice administered with a Low-fat diet, furthermore, species from Clostridiales order were observed which is negatively associated with hepatic cholesterol ester level. Thus, consumption of red cabbage modulated the altered lipid metabolism in obese mice models through modulation in the gut microbiota (Wu et al. 2023a, b). Li and co-workers demonstrated that the administration of broccoli microgreen juice (BMJ) to the obese C57BL/6J mice model resulted in decreased Hb1Ac levels, white adipose tissue mass, and adipocyte size, while modulating the gut microbiome such as decreasing F/B ratio and enhancing the *Bacteroides acidifaciens* at species level. These changes can be negatively correlated with the Lipopolysaccharide (LPS) levels, exhibiting its anti-inflammatory potential. In a nutshell, the consumption of BMJ, exhibits mitigation of diet-induced obesity, by

interfering with gut microbiota-SCFAs-LPS-inflammatory axis (Li et al. 2021). Similarly, in another study, it was demonstrated that broccoli microgreens consumption amended the symptoms of type 2 diabetes in streptozotocin-induced mice by improving the total lipid profile, antioxidant status, and inflammatory factors followed by their interference in the production of propionic acid, and regulation of gut microbiota (Ma et al. 2022). Mohamed et al. reported consumption of barley microgreen alleviated factors associated with type 2 DM and biomarkers of oxidative stress in the streptozotocin and aflatoxin-induced murine model (Mohamed et al. 2022). Similar results were observed after the consumption of clover greens as reported by Aly and co-workers (Aly et al. 2023). *In vitro* anti-diabetic study of R. *sativus* displayed the inhibitory activity of the microgreen against alpha-amylase and is verified by in silico method (Dhanalakshmi 2022). Likewise, Mohammed and co-workers demonstrated the mitigation of factors associated with type 2 DM and biomarkers of oxidative stress in the streptozotocin and aflatoxin-induced murine model by radish microgreen. Both studies demonstrate the potential effect of radish microgreens in reducing the ill effects associated with T2DM and its positive correlation with the gut microbe (Mohamed et al. 2023).

Recently, Legume microgreens have received much attention due to their organoleptic, nourishing, and gut microbiome-modulating effects. Various legumes such as green peas, lentils, mung bean, black sesame, and so on have prolific sets of bioactive components such as polyphenols, alkaloids, chlorophyll, anthocyanin, ascorbic acid that mitigate oxidative stress by enhancing its putative role as a functional food (Kowitcharoen et al. 2021; Altuner et al. 2022).

6 Recent Reports on Legume Microgreen as a Multifaceted Functional Food

Contemporary researchers have conducted multiple studies to explore the various activities of leguminous microgreens. Truzzi et al. demonstrated that when cultivated under fluorescent lighting or a combination of LED various microgreens such as *Pisum sativum*, or pea microgreen have exercised anti-proliferative, anti-tumorogenic, and pro-apoptotic effects against the Ewing sarcoma lines *(*RD-ES and A673) without exhibiting any cytotoxic effects on normal fibroblast cell line (L929) as compared to other microgreen such as *Glycine max*, *Raphanus sativus*, and *Eruca sativa* microgreens (Truzzi et al. 2021). In another study under the influence of different light spectra, Zhang and coworkers exhibited an elevation in the total phenolic and flavonoid content of soybean microgreen as compared to the control. These results establish that under exposure to varied light spectra, the quality of microgreens can be improved and can be utilized as a nutrient supplement to improve the quality of human life (Zhang et al. 2019). Kurian and Megha conducted a comparative study between raw seeds, sprouts, and microgreens of *Vigna radiata* (L.) *Wilczek* (mung bean) and *Cicer arietinum* L. (Chickpea). Microgreens were observed to contain high levels of phenol, and vitamins and exhibited significant free radical scavenging activity as assessed by DPPH (2, 2-diphenyl-1-picrylhydrazyl) (Kurian and Megha 2020). Similarly, a study carried out in Sri Lanka by a group of researchers showcased the gradual increase in the levels of phenols and flavonoids in pea and sesame microgreens as compared to seeds and sprouts that can be positively correlated with antioxidant activity (DPPH) (Senevirathne et al. 2019). In a

controlled condition, the mung bean microgreens showed elevated levels of total phenol and total flavonoid levels which might have influenced the increased radical quenching and total antioxidant activity as evaluated by DPPH and FRAP (Ferric reducing antioxidant power) assays (Goswami et al. 2022). In another study, varied genotypes of mung bean and lentils have displayed elevated levels of total phenol and flavonoid content (TPC and TFC) when grown at high altitudes as compared to plain altitude. The elevation in TPC by mung bean can be positively correlated with FRAP and DPPH while the elevation in TFC by lentils can be positively correlated with DPPH (Priti et al. 2021). Similarly, Kaur and co-workers grew *Vigna mungo* (black gram), *Vigna radiata* (*L.*) *Wilczek* (mung bean), and *Cicer arietinum L.* (Chickpea) under different growing conditions such as soil, water, and cocopeat with nutrients (Kaur et al. 2022). Biofortification of microgreens can be an ultimate strategy to combat malnutrition. Klopsch and co-workers fortified the bread with pea microgreen and observed no substantial decrease in quercetin in processed products as compared to non-processed (Klopsch et al. 2018). Likewise, Kaur et al. prepared eggless rice muffins incorporated with mungbean microgreen powder at different concentrations. It was observed that as compared to rice muffins, mungbean-incorporated muffins exhibit high dietary fiber, and high flavonoids with significantly elevated antioxidant levels. Therefore, pea microgreens and leaves as well as mung bean microgreens are appropriate, natural ingredients for boosting the secondary plant metabolites in bread and eggless muffins that promote health, and can even be utilized to customize bread/muffins to meet the health needs of individual consumers.

7 Limitations and Future Perspective

Although various studies have been carried out on microgreens. Very few studies on legume microgreens have been undertaken. Though a substantially high amount of phenolic, flavonoid, vitamin, and other nutrients are reported from legume microgreen, no considerable *in vivo* and in silico studies are performed to validate the claim.

Few studies have focussed on the role of microgreens on cancerous cells and hence proved to be effective as an anti-tumorogenic natural product. However, the underlying mechanisms and how they affect a living system still need to be proven.

Microgreens are proven to exhibit weight lowering ability, reduction of adipocyte size and number, improve insulin sensitivity, and maintain glucose homeostasis in the body. Though no considerable studies w.r.t microgreens have been carried out in legume microgreens.

Further to prove the putative role as nutrient supplement, legume microgreen needed to be provided to large population, and their effects in the betterment of life style needed to be observed and validated.

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8 Conclusion

Research based on evidence is necessary, and there is much more to learn about the application of microgreens as personal medicine. To improve health by lowering prevailing disorders, appropriate knowledge of the nutritional qualities, preparation techniques, sensory qualities, and palatability of the microgreen communities should be provided. More research is necessary to fully understand the individual and combined health benefits of microgreens.

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Leguminous Microgreens as a Sustainable Source of Essential Nutrients

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Abbreviations

Ahr Aryl Hydrocarbon Receptor
CCP Critical Control Point
CEE Controlled Environment Ecosystem
COX-2 Cyclooxygenase-2
CT Condensed Tannins
CVD Cardiovascular Disease
DIM 3,3-Diindolylmethane
HDL High Density Lipoprotein
LDL Low-Density Lipoprotein
LDPE-SSB Low-Density Polyethylene Self-Seal Bag
LED Light Emitting Diode
MAP Modified Atmosphere Packaging
NASA National Aeronautics and Space Administration
NF-κB Nuclear Factor Kappa Light Chain-Enhancer of Activated B Cells
PET-CS Polyethylene Terephthalate C-Tracer Synthesizer
PTTG1 Pituitary Tumor-Transforming Gene 1
TOP2A Topoisomerase IIα

1 Introduction

Fruits and vegetables are rich sources of micro and macronutrients, providing an essential reference. Maintaining and improving the dietary value of meals has been a primary concern for consumers, regulators, and researchers to preserve food security and quality. The number of malnourished people is increasing worldwide due to a lack of micronutrients (Estrade et al. 2017). Microgreens are introduced to combat this situation. San Francisco first conceptualized the microgreens in the late 1980s, and they are currently well-known as cutting-edge, innovative culinary components in the best eateries and gourmet food outlets. The term “microgreens” was first introduced in 1998 (Bliss 2014). These are the consumable seedlings collected after fertilization between 7 and 21 days after the first true leaves arise, derived from various plant species (Di Gioia and Santamaria 2015). Microgreens are used as a garnishing agent and a significant ingredient for food preparation (Renna et al. 2017). Microgreens have significantly more potent flavor-enhancing qualities and a wider color and leaf form variation. Recent studies concluded that microgreens have low nitrate levels, a short cycle of cultivation, and higher concentrations of minerals and micronutrients (Xiao et al. 2012; Di Gioia et al. 2023).

Microgreens are gifted crops as they are rich in essential nutrients (Kyriacou et al. 2017). It possesses different kinds of attractive colors, flavors, and consistency that allow its usage in garnishing to enhance the appearance of various dishes (Xiao et al. 2012). They are also known as “functional foods” as they have the capability of disease-resistant and health-gaining properties. Various microgreen families include Apiaceae, Asteraceae, Amaranthaceae, Brassicaceae, Chenopodiaceae, Leguminaceae, and Lamiaceae. They are primarily grown in soilless systems and managed ecosystems using artificial light; hence, they are less vulnerable to pathogens, pest attacks, and climatic factors that increase the productivity of the crop and their properties and cause the collection of secondary metabolites (Ying et al. 2020; Enssle 2020). Microgreens fill the nutritional gap; hence, they are recognized as “superfoods” for upcoming generations (Kamal et al. 2020; Pinto et al. 2015). However, microgreens low production, quick aging, and limited longevity prevent their commercial cultivation from growing (Kou et al. 2013; Choe et al. 2018).

The quality of microgreens relies on handling conditions; hence, it is critical to use agricultural techniques while working with these fragile food items (Samuolienė et al. 2013). Microgreens are eco-friendly and need less energy than mature vegetables (Alley et al. 2007; Fuentes-

Peñailillo et al. 2024). Microgreens are an excellent food that is added to a regular diet, recycled, grown quickly without relying on the weather, and transported in less than a week to feed impoverished areas. Different chemical treatments, controlled temperatures, altered atmospheres, light intensities, and pressure have all contributed to preserving the quality of nutritional value and prolonging the shelf life. Due to the presence of anti-oxidants, microgreens act as the scavenging molecule that neutralizes the free radicals upon exposure to oxidative stress. Possible foods from plants and diets rich in vitamins, minerals, fibers, and anti-oxidants, such as microgreens, can lower the risk of several types of cancer and heart disease. The microgreens can be kept for 10 days at a regulated temperature and humidity level in an incubator or climate chamber at 5 °C in polypropylene bags; the biomass of microgreens is increased with the help of biostimulants (Dembele 2021). Compared with microgreens cultivated in non-fertilized media, those grown in fertilized press are more likely to experience contamination from microbial sources. As a result, harvesting should be done within 120 days post-application of non-composted manure, as suggested by the Food and Agricultural Organization (Reed et al. 2018).

People may experience enhanced wellness and higher-quality nutrition by adding microgreens to their eating habits. A controlled environment ecosystem (CEE) plant growth chamber cultivates microgreens and other horticultural crops in a regulative ecosystem. Crops and microgreens are grown using eco-friendly technologies like aeroponics, aquaponics, geoponics, aquaculture, and hydroponics in shielded farming, vertical agriculture, container farming, plant factories, and the biomass production chamber of the National Aeronautics and Space Administration (NASA). Due to its ability to be quickly grown on less expensive substrates in areas with restricted water and land availability, microgreens are becoming more and more environmentally friendly, offering more cost-effective options for producing crops in small spaces.

Due in large part to their improbable nutritional value for thousands of people, particularly in developing countries, legumes are an essential part of the human diet. 2016 was declared the International Year of Pulses by the Food and Agriculture Organisation of the United Nations to raise awareness of the health benefits of pulses, their ecological value, and how they can adapt to climate change as part of a workable food production strategy that aims to achieve sustainable food supply and adequate nutrition. They have anti-bacterial, anti-inflammatory, anti-cancer, and anti-oxidant properties. Due to their high carbohydrate, protein, dietary fibers, and high energy source food content, legumes play a significant role in nutrition in many parts of the world (Bubelova et al. 2018). Furthermore, legumes are rich in secondary metabolites, including flavonoids, carotenoids, green leaves, and anti-oxidants that protect

against various diseases. These substances, which the body cannot produce, can compensate for dietary deficits. Niroula, 2019 states that numerous studies have been conducted on cereals' bioactive and antioxidant properties, including corn, peas, wheat, and barley (Niroula et al. 2019). The biochemical capabilities of several legume species, like Onobrychis, Trifolium, and Medicago species, still need to be discovered despite their widespread consumption, as the study primarily focuses on aromatic and medicinal plants (Kabtni et al. 2020) (Fig. 1).



Fig. 1 Cultivation of microgreens. (Image credit: Idéalités, CC BY-SA 4.0 https://creativecommons.org/licenses/by-sa/4.0, via Wikimedia Commons)

2 Microgreen's Current Status in the World

Many regions of the world's farming industries are dealing with food insecurity due to the Covid-19 outbreak. Diet-related illnesses, such as diabetes, obesity, hypertension, cancer, and others, are becoming more common in both developed and developing nations. This is partially because food intake is unbalanced and typically falls below prescribed amounts (Choe et al. 2018). Additionally, there was a greater need for ultra-processed food and fresh greens provided by Urban Agriculture (UA). Due to its recognition as a crucial instrument for maintaining local food security and generating revenue and employment in metropolitan areas, urban agriculture (UA) has grown considerably in popularity recently (Lal 2020; Paraschivu and Cotuna 2021). The primary objective of sustainable development is to eliminate hidden global hunger,

malnutrition, and food shortages. By 2030, we should strive to attain Zero Hunger and improved nutrition (White and Broadley 2009). Microgreens are a unique food product that provides a diverse range of nutrients such as vitamins, antioxidants, minerals, and other secondary metabolites. Also, the production of microgreens by Urban Agriculture is an important part. Amaranth, Radish, Cabbage, Celery, Mustard, Parsley, Chard, Cress, Fennel, Kale, Arugula, Chervil, Cilantro, Beets, Basil, and Sorrel are among the most popular microgreen kinds. Microgreens can also occasionally be grown from legumes like beans, lentils, chickpeas, and cereals like rice, oats, wheat, corn, and barley. The global market for microgreens is expanding due to their many health advantages, rich in nutrients, and the quick uptake of indoor growing in cities (Pattnaik et al. 2020). Since microgreens are 40 times richer in nutrients than mature veggies, they are becoming increasingly well-liked worldwide for their beneficial benefits on human well-being and appearance (Muchjajib et al. 2014). With 71% and 59% profitability in 2020, the North and South of the United States had the most significant rates of microgreen production in greenhouses. However, consumer behavior and income level will determine whether the microgreens market develops to its full potential. As a vegetable substitute, microgreens could lessen the worldwide food shortage and the difficulties associated with malnutrition (Fig. 2).

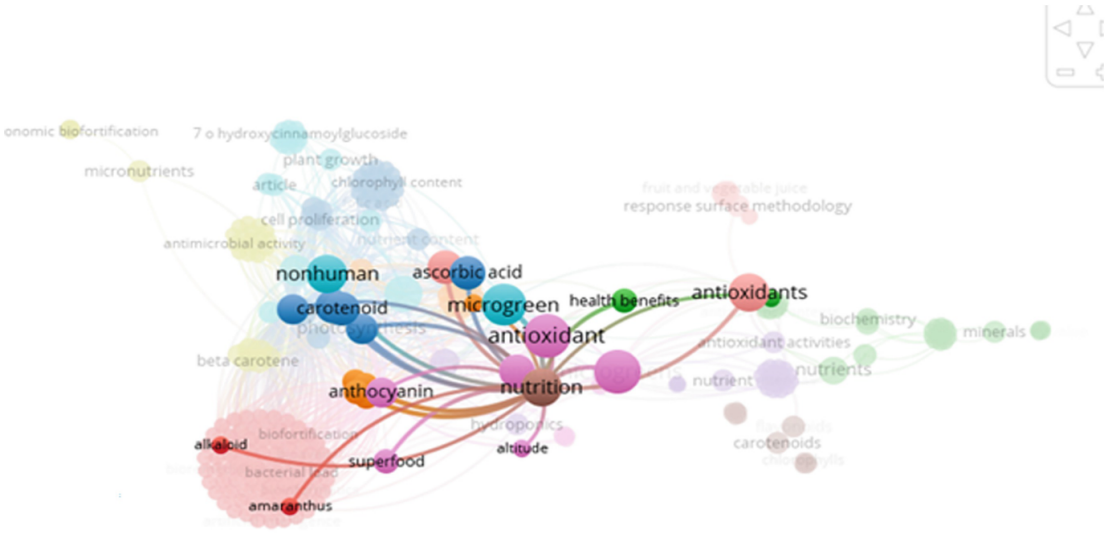


Fig. 2 In this bubble map, the microgreen bubble is connected with keywords like anti-oxidants, health benefits, superfood, and amaranthus as they are correlated with one another. The size of each bubble represents the number of citations for that keyword. Different colors of each bubble represent publications from different journals. Interaction between any two bubbles represents the connection. This bubble map is made using the software VOSviewer (Version 1.6.19)

3 Pre- and Post-harvest Interventions

It has been demonstrated that pre- and post-harvest manipulations influence microgreen's dietary value. Various pre- and post-harvest therapies seek to reduce the risk for safety, eradicate the bacteria, and increase the productivity of the microgreens. Several pre- and post-harvest procedures, including adjustments to growth conditions, preharvest treatments, appropriate harvesting, adjustments to the storage environment and transportation circumstances, as well as an addition to producing more durable, palatable, and beneficial products, are crucial factors to take into account for optimal microgreens utilization (Paradiso et al. 2018).

3.1 Pre-harvest Measures

3.1.1 Light Conditions

For example, lighting setup to microgreen enhance the nutritional value (Wang et al. 2017). Mlinaric et al. demonstrated that chia microgreens have high bioactive compounds in the presence of light instead of in dark conditions (Mlinarić et al. 2020). Hence, it seems that light before harvest plays an essential role in the accumulation of nutrients. Kopsell et al. stated that high concentrations of photosynthetic pigments are accumulated in LED treatment groups instead of fluorescent treatment groups (Kopsell et al. 2017). In the presence of UV-A light, the accumulation of secondary metabolites in microgreens is much greater than in LED and normal light. Microgreens possess high anti-oxidant and free radical scavenging activity in the presence of UV-A light compared to regular light (Zhang et al. 2019).

3.1.2 Biofortification and Nutrients of the Plant

Apart from light sources, several other factors, like the addition of minerals and vitamins in the microgreens, natural substrates, heat, and salinity stress, play an essential role in enhancing the anti-oxidants and nutritional value of the microgreens. Microgreens have a high rate of respiration during harvesting. To increase nutrient content for storage in broccoli microgreens and delay senescence, it is suggested to add calcium chloride prior to harvesting (Kou et al. 2014). The combined effect of calcium chloride and UV-B as pre- and post-harvest interventions increases the microgreens' overall quality, anti-oxidants, shelf-life, and productivity (Lu et al. 2018). Pre-harvest measures could significantly improve sprouts' microbial security even in the lack of a verified Critical Control Point (CCP) meant to bring fecal pathogen levels down to tolerable levels (Galieni et al. 2020; Symes et al. 2015). More research is needed on the sterilization procedures for microgreens before and after harvest, especially to examine the relationship between morphological traits and sanitizer effectiveness. The main ingredient in broccoli microgreens is glucosinolates, enhanced by preharvest treatment with

10 mM calcium chloride, which also lengthened their shelf life. Preharvest (10 mM calcium chloride) and post-harvest (UV-B irradiation) therapies both extend the total concentration of glucosinolate and increase the shelf life of microgreens (Lu et al. 2021).

3.2 Post-harvest Quality

Proper storage of fresh-cut microgreens depends on factors such as temperature, light exposure, air composition, and packing methods. Furthermore, processing methods, including cooling, heating, waving, heating in the microwave, deep-frying, toasting, and cooking, are equally necessary to preserve the accessibility of the medicinal plants and bioactive components of microgreens while developing value-added products. Nano-packaging technology has also been introduced to enhance the shelf life of microgreens for the management of post-harvest conditions. Despite their relatively low temperatures, microgreens' young tissues, which have a higher respiration rate than their mature counterparts, cause a more rapid loss of quality after harvest. Microgreens' post-harvest quality may be impacted by their genotype, growing season, culture techniques, and harvesting schedule since these variables significantly influence the build-up of non-enzymatic antioxidant chemicals (Bonasia et al. 2019). The preservation of microgreens' post-harvest shelf life depends on several factors, including harvest time, harvesting technique, water vapor transmission rate, cleaning treatments, packaging, storage conditions, respiration rate, oxygen transmission rate, and more.

3.2.1 Handling

Reducing damages is another post-harvest facet that may favorably impact the microgreens' shelf life. Harvesting and post-harvest handling circumstances conditions at the time of growth significantly affect the microgreens' general efficacy. As a result, it is crucial to guarantee that proper agronomic procedures are followed when handling these fragile culinary items (Samuolienė et al. 2013).

3.2.2 Packaging Technology

Variations in the materials used for packaging, pre-and post-harvest procedures, and MAP (modified atmosphere packaging) control how long fresh-cut microgreens stay on the shelf and their sensory qualities. For example, in roselle and radish microgreens, self-seal bags of low-density polyethylene and packing with macro-perforations are used to increase the shelf life (Ghoora and Srividya 2020).

Microgreens can be harvested and stored for extended periods if kept cool and in an unhumidified bag or container. It will prevent them from drying out or wilting while being transported (Turner et al. 2020). Several

strategies have been employed to increase the microgreen's shelf life. Packaging is the most significant element influencing the microgreens' quality and shelf life after temperature. Altered environmental packaging that increases carbon dioxide regulates partial pressure and reduces oxygen, which can also prolong the shelf life of microgreens (Kim et al. 2004). The packaging and materials used to store the items helplower the risk of biological contamination. It is necessary to utilize different packaging materials to preserve and transport freshly harvested microgreens while minimizing any changes in their quantity and quality during the process. By utilizing modified atmosphere packaging (MAP), it is possible to extend microgreens' shelf life by decreasing their transpiration, oxidation, and respiration rates. The produce's freshness can be preserved longer with MAP, increasing its worth during marketing. A study by Dalal and Siddiqui (2019) shows that sunflower microgreens' post-harvest quality and storage life increase using polystyrene trays for packaging compared to low-density polyethylene bags.

The nutritional value and shelf life of roselle and radish microgreens stored in LDPE-SSB and PET-CS at 5 °C and found that the PET-CS experienced less leakage of electrolytes, rate of respiration, weight loss, and microbiological counts than the LDPE-SSB (Ghoora and Srividya 2020).

3.2.3 Storage Conditions and Temperature

Improving postharvest shelf life can be achieved primarily through temperature during storage and the surrounding environment. Temperature is a critical factor that helps increase microgreens' shelf life (Xiao et al. 2014).

The seedlings' development stage at harvest may impact the shelf life of microgreens (Berba and Uchanski 2012; Signore et al. 2024). Research indicates that while baby spinach can be collected at any time during the winter, it is best to harvest it early in the spring to preserve its increased water content, solid texture, and appealing appearance (Garrido et al. 2016).

Efficient temperature control during storage can optimize the shelf life of microgreens. This helps in transit and prevents spoiling while maintaining their nutritional value and keeping them fresh for extended periods. The respiration rate and microbial activities decrease at low temperatures, which can cause deterioration or contamination of the food items (Oliveira et al. 2015). Proper temperature control is essential since elevated storage temperatures typically accelerate respiration and metabolic processes, promote the growth and reproduction of microbes, lead to the production of off-flavors, and hasten the start of aging in packaged goods. Conversely, the low storage temperature may negatively impact the quality of the cold-sensitive microgreens (Oliveira et al. 2015).

Because the rate of chemical reactions almost doubles with every 10 °C increase in temperature, the ability of the microbiota to harm the microgreens may be correlated with the temperature during postharvest storage (Oliveira et al. 2015). Low temperatures are considered better for postharvest storage of microgreen produce because they often limit a cell's ability to metabolize, which keeps most of its enzymes dormant.

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4 Phytochemicals and Bioactive Compounds of Microgreens

Microgreens contain phytochemicals; thus, they act as an anti-inflammatory, anti-microbial, anti-oxidant, and anti-carcinogenic. The bioavailability of these bioactive chemicals in food and herbs influences their health-related qualities, content, and amount ingested.

One of the most prominent families of flowering plants, the Fabaceae, contains about 18,000 species grouped into 650 genera. These species are essential to almost every terrestrial ecosystem on every continent. Grain legumes, often known as pulses, are becoming essential parts of primary and multifunctional meals due to their high nutritional content, richness of minerals, and secondary metabolites (Bouchenak and Lamri-Senhadji 2013).

Microgreens contain low amounts of nitrate and oxalic acid, as both are antinutrients that cause a reduction in the absorption of minerals.

Giménez et al. (2021) and El-Nakhel et al. (2021) claim that growing practices and surroundings have a crucial effect on the accessibility of nutrients in microgreens.

Microgreens contain large amounts of fatty acids, bioactive compounds known as plant chemicals, amino acids, proteins, and carbohydrates and are thought to be low in antinutrients (Benincasa et al. 2019; Sharma et al. 2022). Microgreens are rich in vitamins, phytonutrients (ascorbic acid, phylloquinone, β-carotene, and α-tocopherol), anti-oxidants, minerals (Ca, K, Mg, Zn, Na, Mo). They are also regarded as a good source of biologically active substances that prevent long-term illnesses and nutritional deficiencies (Xiao et al. 2016; Singh et al. 2021).

Legumes such as peas and lupin are rich in flavonoids, chlorophylls, and carotenoids, which are examples of secondary plant metabolites that benefit health. The predominance of quercetin glycosides indicates that pea microgreens and leaves are both excellent sources of quercetin. "Lupin microgreen bread" containing a considerable amount of genistein may benefit health, especially for women. High amounts of anti-oxidants and phenolic compounds are present in red clover. Maize contains a large amount of flavonoids. Lentil contains a vast amount of chlorophyll, chlorophyll a, chlorophyll b, and carotenoid compared to Mung bean. Because of their low glycemic index, pulses are a healthy dietary option

for anyone with diabetes following a regulated diet. Lentils are also high in fiber, protein, and complex carbohydrates (Gan et al. 2017). Lentil microgreens are rich in carotenoids, ascorbic acid, and chlorophylls.

Nutritional supplements made from mungbean sprouts are used in various medical fields. Compared to their mature counterparts, microgreens have a substantially higher phylloquinone content. Because of their nutritious qualities, several cereals (corn, oats, barley, and wheat) and legumes (beans, lentils, and chickpeas) can also grow and manufacture microgreens. Carotenoids are present in barley and wheat microgreens, making them a rich nutrient source (Niroula et al. 2019). Microgreens contain vitamins and other active phytochemicals, including carotenoids, tocopherols, glucosinolates, phenols, and betaine, which help maintain health (Kyriacou et al. 2019).

5 Legume-Derived Indigenous Foods

As members of the Fabaceae/Leguminaceae family, legumes are the third most prominent family of angiosperms (Gepts et al. 2005). This family comprises peas, lentils, beans, peanuts, and other edible pod plants. They offer a variety of vital elements, including protein, carbs with a low glycemic index, dietary fiber, minerals, and vitamins. A variety of foods are prepared with the help of legumes, as shown in Table 1.

Table 1 Some legume-derived foods

Legume/substrate (common name)	Scientific name	Bioactive compounds	Product	Country	References
Cowpea	*Vigna unguiculata*	Polyphenols and peptides	Akara	West Africa	Kethireddipalli et al. (2002)
Black gram	*Vigna mungo*	Polyphenols, carotenoids	Wadi/bori/wari	India, Bangladesh, and Pakistan	Yadav and Khetarpaul (1994)
Chickpea	*Cicer arietinum*	Phenols, carotenoids, tocopherols	Dhokla	Western India	Hirdyani (2014)
Soya bean	*Glycine max*	Isoflavones, saponins	Yandou	China	Qin et al. (2013)
Soya bean	*Glycine max*	Phytic acids, phytosterols	Thua nao	Thailand	Chukeatirote (2015)
Peanut	*Arachis hypogaea*	Flavonoids, phenolic compounds	Peanut butter/Pindakaas	Suriname	Hoekstra and van der Kuip (2017)
Black gram	*Vigna mungo*	Phenolic acids like gallic, protocatechuic, gentisic, vanillic,	Papad	India, Bangladesh, and Pakistan	Gaikwad et al. (2022)

Legume/substrate (common name)	Scientific name	Bioactive compounds	Product	Country	References
		syringic, caffeic, and ferulic acids			
Broad bean	*Vicia faba*	Phenols, flavonoids, and tannins	Nabid/Nabit/Nabet	Egypt	Elbadrawy and Mostafa (2023)
Velvet bean	*Mucuna pruriens*	Phenolics, tannins, L-Dopa, and phytic acid	Benguk	Philippines, Indonesia, Ghana, Malawi and Nigeria	Das et al. (2022)

6 Beneficial for Health

The consumption of microgreens has increased due to the presence of secondary metabolites, minerals, and vitamins to improve human health. The most significant advantage of microgreens is that they can be consumed raw, which minimizes the adverse effects of being overcooked, processing of the food, and the deterioration of sensitive B complex vitamins. It increases the absorption of nutrients and bioactive compounds, particularly water-soluble micronutrients and phenolic compounds, which are heat- and processing-sensitive (Ebert 2022).

The leaves of mature vegetables have much lower levels of phytonutrients than microgreens, as studies have found that the latter may contain up to 40 times more beneficial compounds (Xiao et al. 2016). Therefore, the nutrients in microgreens boost appetite the immune system, and prevent Alzheimer's disease, diabetes, and muscle degeneration illness, lower the chance of a heart attack, are beneficial for skin and eyes, and enhance the body's total hormonal balance (Huang et al. 2016; Wadhawan et al. 2018). Evidence from history indicates that certain types of evergreen legumes and pulses were utilized as food or medicine in prehistoric times. Around the globe, red clover is commonly planted as a crop for cattle and poultry and is utilized in traditional medicine. *Alfalfa* is a significant feeder crop grown worldwide. It has long been used in traditional herbal medicine to cure various illnesses in China, Iraq, Turkey, India, and the United States (Bora and Sharma 2011; Durairajan et al. 2024).

Condensed tannins (CT) are present in sainfoin plants. In human medicine, these substances have drawn attention to their potential as anti-oxidants and antibiotics (Cos et al. 2004). The amount of isoflavones is high in zigzag clover due to which they are frequently utilized as sources of phytoestrogens (Seguin and Zheng 2006; Kazlauskaite et al. 2024), which led to the choice to involve a wild ecotype of zigzag clover in the research.

Black medic is a multipurpose legume used worldwide in temperate and subtropical climates for green manure, pasture, cover crops, intercropping, phytoremediation, and medicinal use (Agelet and Valles 2003).

It has been reported that pea/lupin microgreens are an excellent natural component for bread that contains quercetin and genistein. Therefore, traditional foods enhanced with microgreens may satisfy customers' unique health needs and offer supplemental beneficial nutrients.

Plants contain phenolic chemicals (anthocyanin, rutin, isoflavone, and quercetin), comprising a diverse category of secondary metabolic products. They are essential to the human diet and give fruits and vegetables their color, flavor, and perfume. Numerous studies have emphasized phenolic-rich diets' antimicrobial, anti-inflammatory, anti-adhesive, and anti-oxidant qualities and their potential to improve health (Ferreira et al. 2017). Flavonoids are a phenolic compound commonly found in fruits and vegetables, often in high quantities.

Chlorophyll and carotenoids are two examples of the pigments produced by photosynthesis that microgreens invariably produce throughout the process of photomorphogenesis. Chlorophyll is plants' most noticeable and common pigment since photosynthesis depends on it. Furthermore, it has been demonstrated that chlorophylls are essential for maintaining human health because of their strong anti-oxidant and anti-inflammatory effects (Lee et al. 2017).

Phylloquinone has been found in certain microgreen species and is essential for blood coagulation and bone repair (Xiao et al. 2019).

β-Carotene is an anti-oxidant crucial in developing vision and is found in microgreen species (Kyriacou et al. 2016).

Microgreens are rich in carotenoids, which are found in the retina and lens of the eye and function as anti-oxidants and UV protection for the eyes (Xiao et al. 2019).

The tiny-seeded legumes offer unlabeled sources of possible components for adding nutrients, minerals, and bioactive chemicals to traditional or essential cuisines (Fig. 3).

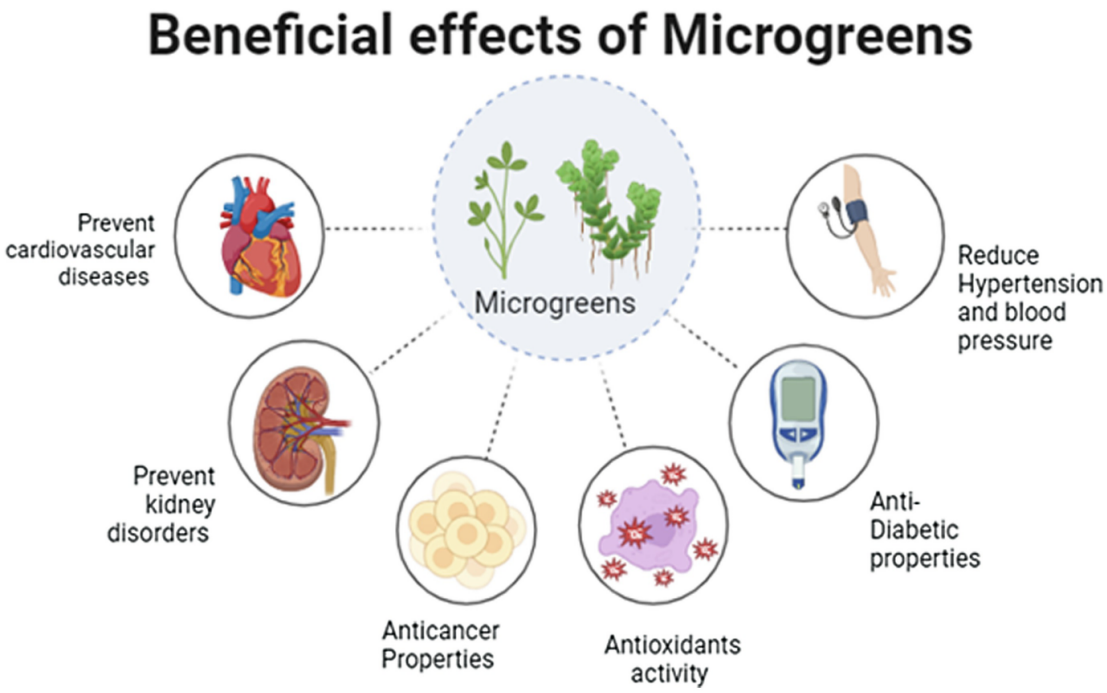


Fig. 3 Beneficial effects of microgreens. The images are made using BioRender software (https://www.biorender.com/)

7 Regulation of Health and Functional Significance

In recent years, microgreens have gained popularity as nutritious dietary additives because of their extremely high nutrient content, which includes both macro and micronutrients with important physiological roles. Epidemiological research states that consuming vegetables and fruits prevents chronic diseases from rising yearly. There are few studies on the health benefits of microgreens in vivo, their efficacy in controlling blood sugar and weight, and their ability to regulate adipose tissue provide the groundwork for future research on the potential benefits of microgreens in preventing and treating chronic diseases, including obesity, cardiovascular diseases, and type 2 diabetes (Wadhawan et al. 2018; Huang et al. 2016).

7.1 Microgreens' Anti-Inflammatory and Immunomodulatory Effects

Many long-term diseases, such as obesity, cardiovascular disease (CVD), and cancer, are primarily attributed to inflammation in its genesis, growth, and advancement. Therefore, controlling inflammation can broadly affect managing healthcare expenses and preventing several diseases.

Some research has shown that the presence of glucosinolates and other phenolic compounds, such as flavonoids and hydroxycinnamic acid derivatives, can disrupt different signaling pathways, such as the one that

NF-κB (nuclear factor kappa light chain-enhancer of activated B cells) uses to trigger the transcription of pro-inflammatory genes, which in turn controls inflammation. The role of free radicals in the negative regulation of inflammatory pathways has been thoroughly investigated. Reactive oxygen species can affect NF-κB pathways and other signaling pathways linked to inflammation, but the antioxidants in microgreens effectively suppress these effects.

Microgreens possess polyphenols, which help inhibit COX-2, as they can disturb the process of inflammation by producing prostaglandin (Subedi et al. 2019).

Microgreens have a high concentration of AhR ligands in their phytochemical composition. It suggests that glucosinolates and other polyphenols, including indole-3-carbinol, can affect AhR-mediated immune reactions and regulation of T-cells (Subedi et al. 2019; Wheeler et al. 2017).

7.2 Microgreens' Anti-diabetic, Anti-obesity, and Anti-cardiovascular Effects

The literature published in the last decade and the experimental findings emphasize that consuming a fruit- and vegetable-rich diet should be the first line of defense and genuine recommendation for preventing chronic health diseases (Ülger et al. 2018). Recent research has shown that microgreens may regulate the breakdown of lipids and adipogenesis simultaneously, which may serve as a protective mechanism against obesity, diabetes, cardiovascular disease, and obesity-related co-morbidity. Due to their high nutritional value and presence of active components, microgreens gained popularity for ingesting food and drinks that protect against various types of chronic diseases. Research has demonstrated that dietary fiber polyphenols and glycosinolates can lower cholesterol levels (Złotek et al. 2019). Consumption of microgreens substantially controls the plasma level of low-density lipoprotein, formation of hepatic cholesterol ester, and concentration of triglycerides by lowering the production of inflammatory cytokines in the liver. Huang conducted an in vivo experiment to determine microgreens' effectiveness in lowering cholesterol and fat levels. They used a high-fat diet to create an obesity model and added microgreens to their meals (Huang et al. 2016). The mice shed pounds and markedly reduced triacylglycerol levels, cholesterol ester, inflammatory cytokines, and LDL formation in their livers (Wojdyło et al. 2020). Baked beans and sweet lupin are examples of legume microgreens that help treat hypercholesterolemia (Winham and Hutchins 2007; Arnoldi et al. 2013).

Moreover, the study indicated that a decline in hepatic inflammatory and cholesterol cytokines levels was associated with the suppression of lecithin-cholesterol acyltransferase, glycerol-3-phosphate acyltransferase,

and acetyl-CoA acetyltransferase-3 enzymes responsible for triglyceride synthesis. Numerous microgreens contain high levels of Indole-3-carbinol, which, when activated, stimulates cellular signaling linked to the regulation of thermogenesis, mitochondrial oxidation of fatty acids, inhibition of inflammatory cytokines, and uptake of glucose, all of which reduce the risk of developing various cardiovascular diseases (Zhang 2007). Fenugreek microgreens contain anti-oxidants and polyphenols that have anti-diabetic effects, thus enhancing glucose uptake (Wadhawan et al. 2018).

7.3 Microgreens' Anti-cancerous Activity

The development of cancer, its spreading, and its effective treatment remain evasive as it changes with changes in circumstances. Regular consumption of a healthy diet helps prevent approximately one-third of cancers. Microgreens may defend against or avert cancer by potentially influencing pathways associated with inflammation. Certain microgreens contain relatively high concentrations of several diet-derived chemicals, including DIM (3,3-diindolylmethane) and indole-3-carbinol, which have been shown in multiple studies to activate enzymes involved in the metabolism of xenobiotics (Kim and Milner 2005). Soybean and various legumes help to fight against colon and cervical cancer (Robles-Ramírez et al. 2012; Feregrino-Perez et al. 2008; Shea et al. 2024). It is unclear which of the many oncogenic pathways are under control, and not many clinical studies demonstrate the anti-cancer effects of dietary polyphenols. Further research is necessary to explore the possibility of microgreens having the potential to prevent cancer.

7.4 Modulation of the Gut Microbiome by Microgreens

While changes in the gut microbiome can occur instantly, a consistent dietary pattern is essential for its modification. Microgreens are rich in flavonoids such as quercetin, kaempferol, and apigenin, which help modulate the gut microbiome and protect against various diseases.

Various kinds of legumes have been reported to treat metabolic disorders, as represented in Table 2.

Table 2 Various health benefits of legumes

Sources	Metabolisms/diseases	Beneficial effect	References
Fenugreek	Diabetes	Increase glucose uptake	Wadhawan et al. (2018)
Baked beans	Hypercholesterolemia	5.6% reduction in HDL-cholesterol and 5.4% in LDL-cholesterol	Winham and Hutchins (2007)
Raw or cooked-bean extracts	Colon cancer	Inhibition of aberrant foci crypt expansion in rat intestine	Feregrino-Perez et al. (2008)
Protein isolates of germinated	Cervical cancer	Reduced expression of TOP2A and PTTG1 triggering apoptosis of	Robles-Ramírez et al. (2012)

Sources	Metabolisms/diseases	Beneficial effect	References
soybean		cancer cells.	
Sweet lupin	Cardiovascular	More significant improvement in HDL-cholesterol and LDL-cholesterol	Arnoldi et al. (2013)
Azuki bean juice	Hypertriglyceridemia	Decline in triglyceride	Maruyama et al. (2008)
Various legumes	Colorectal cancer	Enhance beneficial Gut microflora like Prevotella bacteria and Firmicutes (fiber degrading).	Filippis et al. (2016)

8 Limitations

Growing conditions that promote the development or spread of microorganisms, processing methods that expose the crop to contaminants from humans or animals, and plant characteristics that allow for contact and interaction with germs all pose a risk to the safety of crops. The primary drawback of microgreens is their rapid degradation of quality after harvesting, as they have a large surface area, fragile leaves, small post-harvest shelf life, and high respiration rate (Chandra et al. 2012). Microgreens have a short shelf life and tend to age quickly, making them highly perishable items. According to Chandra et al. (2012), these greens typically last only 3–5 days at room temperature, which limits their marketing opportunities.

9 Conclusion

Microgreens hold great promise for bringing leafy crop cultivation to a microscale, enhancing the nutritional content of the human diet, and influencing dietary patterns. These are nutrient-dense, low-calorie foods that are also abundant in bioactive chemicals. Microgreens are rich in nutrients and thus prevent various kinds of diseases. They are primarily grown in a controlled environment, especially aquaponics and hydroponics. It is simple to grow with the least amount of resources. Their post-harvest life is crucial, and various pre- and post-harvest treatments can improve their durability, nutrient content, and functionality. The major challenge for microgreen cultivation is no quality control or standard operating procedures. Industry-wide guidelines must be set for selecting seeds, growing material, temperature, humidity, lighting, and nutrition management. Market growth will be aided by increasing education and consumer awareness of microgreens' culinary applications, nutritional benefits, and eco-friendly features.

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Updates on Bioactive Compound of Microgreens: A Novel Superfood for Monitoring Lifestyle Disease

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Keywords Antioxidant – Bioactive compound – Chronic diseases – Human health – Metabolic diseases – Microgreens – Superfood

Abbreviations

NC Nutrient Composition
PPH Primary Polyphenols
SFN Sulforaphane
TAA Total Ascorbic Acid
TPC Total Phenolic Content
UA Urban Agriculture

USD United States Dollar

Vit Vitamin

1 Introduction

Over the last decade, people have shown a growing interest in microgreens, for their high nutritional value and enriched bioactive phytocompounds. By including these substances in a balanced diet, people can prevent chronic diseases and encourage overall wellness. Microgreens are small and delicate plants that can belong to a variety of vegetables, herbs, flowers, or grains. Despite their size, they offer intense flavor, texture, aroma, and exotic colours. These functional micro-vegetables are rich in various phytonutrients, which vary depending on the types of plants that are used to produce them (Samuoliene et al. 2019). Microgreens are frequently referred to as the next generation “superfoods” or “functional foods” because of their abundance of nutrients that promote health, including antioxidants, vitamins, minerals, and phenolic compounds. It can be harvested just 1–3 weeks after the cotyledons are fully expanded and turgid when the first pair of leaves appear (Zhang et al. 2021; Jambor et al. 2022). This usually occurs within 7–21 days from seed germination, depending on the species (Sun et al. 2013). It can be grown in various places such as homes, restaurants, schools, etc. Additionally, it can be produced throughout the year in a controlled environment. Along with other carotenoids like violaxanthin and zeaxanthin, microgreens are important sources of vitamins like ascorbic acid, tocopherol, phylloquinone and β-carotene. It can be produced on a bigger scale for commercial production or in smaller quantities for private usage. A variety of commonly grown vegetables, belonging to different families such as Amaranthaceae, Amaryllidaceae, Apiaceae, Asteraceae,

Boraginaceae, Brassicaceae, Convolvulaceae, Cucurbitaceae, Malvaceae, Poaceae, Lamiaceae, Leguminosae, Onagraceae, and Portulacaceae can be used to cultivate microgreens (Ebert 2022; Marchioni et al. 2021; Di Bella et al. 2020; Tan et al. 2019). Microgreens in the Brassicaceae family have a variety of biological properties, including isothiocyanates and glucosinolates that have been shown to protect against cancer. Additionally, they have antioxidant, anti-inflammatory, anti-diabetic, neuroprotective, and cholesterol-lowering effects (Ramirez et al. 2020; Ebert 2022). Secondary metabolism produces the majority of bioactive substances. The most significant ones are glucosinolates. Upon their degradation, indoles and isothiocyanates are produced as byproducts. Additionally, compounds such as phenolic compounds, carotenoids, tocopherols, and ascorbic acid are also of great interest in this family (Ramirez et al. 2020).

Nowadays cardiovascular diseases, neurodegenerative disorders and diabetes, a major health concerns worldwide, largely because of unhealthy diets and lack of physical exercise. However, research indicates that eating microgreens might lower the chance of developing this illness. Specifically, it has been discovered that red cabbage microgreens improve lipid and cholesterol profiles. For example, a study by Huang et al. (2016) found that red cabbage microgreens can help reduce weight gain, low-density lipoprotein, triglycerides, hepatic cholesterol ester levels, and liver inflammatory cytokines in mice. Additionally, recent research on human colon cancer Caco-2 cells has shown that microgreens from the Brassicaceae family have significant anti-proliferative effects on cancer (De La Fuente et al. 2020). This may be due to higher levels of polyphenols and glucosinolates found in microgreens, which are believed to modulate the immune system and prevent the aforementioned diseases. (Lopez-Chillon et al. 2019). Over time, multiple literatures have assessed the

nutritional benefits of microgreens. In one extensive study, it was reported that 25 different commercially available microgreen varieties contained an impressive concentration of essential vitamins and carotenoids (Xiao et al. 2012). According to Marchioni et al. (2021), the essential oils of broccoli, daikon, mustard, rocket salad, and watercress contain a total of 50 different compounds.

The purpose of the current book chapters is to emphasize the significance of microgreens compounds in different lifestyle originated disease and provide in-depth information about the mechanical vision that direct at the molecular level in reducing the physiological barrier and improving the immunity against that disease.

2 Microgreens: Current Status

In the present era, worldwide more than 25 types of microgreens are produced commercially. Over the last decade, trends in the agricultural market have changed dramatically because of adopting new technologies for improving cropping quality and diversification. Ongoing climate changes show negative impacts on sustainable agriculture farming, which impact on socio-economics context of the common population. The greatest challenge between the farmers and researchers are feeding over ten billion common people of the worldwide population by 2050. Urban Agriculture (UA) report showed that in cities moreover 80% of food consumed worldwide by 2050 (Martin-Moreau et al. 2019). UA played a crucial role in the production of microgreens in present day, whereas it was also established that the nutritional compositions are 40 times higher than the mature leaves of vegetables (Xiao et al. 2016). The microgreens market was expected maybe worth 1445 USD, million in 2021, whereas the global scenario of the microgreen market grows annually at 7.6% and reaches 17039.744 USD, million in 2025 (KSI 2020).

Microgreens reaches 60% profitability because of their high revenue in comparison with other common indoor crops. Microgreens have extended at 26% and 10% in large and small farms, respectively in 2017, and in future to be increased to 6% annually (SIFR 2017). Cultivation profitability of microgreens under greenhouse technology in North and South United States regions reached 59% and 71%, respectively (Mordor Intelligence Platform Report 2020). In 2022, the maximum microgreens market found in Asia, Canada, Australia and United States (Fig. 1).



Fig. 1 Microgreens market: market size, by region, 2022. (Source: Mordor Intelligence Platform Report (2022))

3 Chemical Composition and Functional Compounds in Microgreens

Young vegetable greens known as microgreens, are collected at their early life phase. They are renowned for having a high nutrient density, vivid colours, and strong Flavors. These are some common traits, though their

nutritional makeup might vary based on the particular plant type, growing environment, and stage of growth. Soon after the development of the first few leaves, they are harvested. Microgreens are incredibly nutrient-dense and can offer concentrated amounts of antioxidants, vitamins, and minerals despite their tiny size. Microgreens have many kinds of Vitamins A, C, E, K and Minerals. These young greens are a great supply of antioxidants, which help shield the body from oxidative stress and free radical damage. Microgreens are rich in carotenoids, flavonoids, and polyphenols, which are function as antioxidants. The sugar content is very low in microgreens, so it is suitable for diabetes patients. Microgreens are a nutrient-dense supplement to meals without greatly increasing calorie consumption because they are often low in calories. Microgreens are frequently found in foods like broccoli, arugula, radish, kale, cilantro etc. Some of the chemical composition and functional compounds of microgreens are described (Table 1; Fig. 2).

Table 1 List of microgreens and their function and chemical composition

Microgreens names	Microgreens properties	References
Commercial microgreens	The range of total ascorbic acid concentrations per 100 g fresh weight (FW) was 20.4–147.0 mg. The levels of phylloquinone ranged from 0.6 to 4.1 µg/g FW, while the levels of alpha and gamma tocopherol varied from ~5 to 87 and ~3 to 39 mg/100 g FW respectively. The amounts of β-carotene, violaxanthin and lutein/zeaxanthin were found to vary from ~0.6 to 12, 0.9 to 7.7 and 1.3 to 10 mg/100 g FW respectively.	Xiao et al. (2012)
Broccoli (*Brassica oleracea* var. *italica*)	Ascorbic acid application was raised from 0.1% to 0.5%, resulting in a rise in total ascorbic acid.	Kathi et al. (2023)

Microgreens names	Microgreens properties	References
	More specifically, microgreens treated with lower concentrations of ascorbic acid (i.e., 0.05% and 0.1%) in trial 1 and 0.25% ascorbic acid treatment in trial 2 exhibited higher values of chlorophyll a & chlorophyll b, Total chlorophyll, and carotenoids. Furthermore, with minimum ascorbic acid treatments and control, higher amounts of nitrogen, copper, manganese, sulphur, zinc, calcium, iron, and phosphorus were observed.	
Daucus carota L. var. *New Kuroda*, *Foeniculum vulgare* Mill., *Trigonella foenum-graecum* L., *Ocimum basilicum* L., *Brassica juncea* L., *Allium cepa* L. var. *Light Red Gavran*, *Raphanus sativus* L. var. *Imp. Chetki*, *Hibiscus sabdariffa* L. (*white var.*), *Spinacia oleraceae* L. var. *All Green*, *Helianthus annuus* L.	The ascorbic acid level of the microgreens under study varied greatly, ranging from ~30 to 123 mg/100 g. The best microgreens for ascorbic acid were discovered to be roselle, then French basil, fenugreek, and radish microgreens. Radish microgreens had the highest amount of α- tocopherol (58.6 mg/100 g). Microgreens from sunflower, mustard, and fennel were also excellent providers of α-tocopherol. The least amount of α-tocopherol was found in fenugreek microgreens (5.0 mg/100 g). The β-carotene content of the culinary microgreens ranged from around 3 to 9 mg/100 g, with the highest quantity found in fennel, followed by radish and mustard. The microgreens of fenugreek had the lowest concentration.	Ghoora et al. (2020)
Beetroot (*Beta vulgaris*), Mung Beans (*Vigna radiata*), Kale (*Brassica oleracea*), Radish (*Raphanus sativus*), Beetroot (*Beta vulgaris*), Green Peas (*Pisum sativum*), Amaranths (*Amaranthus sp.*).	L-ascorbic acid was highest in broccoli sprouts (~114 mg/100 g FW) and radish sprouts (~94 mg/100 g FW), whereas it was lowest in all other microgreens (~32 mg/100 g FW). It dropped in the following order in microgreens: amaranth > kale > pea (green) > beetroot > radish, ranging	Wojdyło et al. (2020)

Microgreens names	Microgreens properties	References
	from ~1510 to 4073 µg/g FW. Carotenoids in sprouts varied more widely, from ~22 µg/g FW in mung beans to ~949 µg/g FW in radish. All of the examined samples included phenolic acids (ferulic, sinapic, caffeoylquinic) and their derivatives products. Derivatives of isorhamnetin, kaempferol, and quercetin are among the predominant plant flavonols. Microgreens had a chlorophyll content ranging from ~196 to 638 µg/g FW, decreased in the following order seen in amaranth, green peas, beetroot, radish, and kale. The range of sprouts' chlorophyll concentration was from ~6 to 108, with lentils having the highest level.	
Dijon mustard (*Brassica juncea* L. Czern.), Opal basil (*Ocimum basilicum* L.), bull's blood beet (*Beta vulgaris* L.), red Amaranth (*Amaranthus tricolor* L.), eppercress (*Lepidium bonariense* L.) and China rose radish (*Raphanus sativus* L.),	China rose radish has the highest amounts of total ascorbic acid. The highest concentrations of total phylloquinone in Opal basil. Tocopherols concentration highest in China rose radish. Carotenoids highest in red Amaranthus. Red amaranth was the plant with the lowest overall sugar content and the highest pH value, whereas the maximum titratable acidity and total sugar levels were found in China rose radish. Phenolic compound highest in opal basil.	Xiao et al. (2015)
30 distinct microgreen cultivars belonging to the Brassicaceae family	According to the study's findings, in a well balanced diet, Brassicaceae microgreens are an excellent source of macro elements like potassium and calcium as well as microelements like iron and zinc. Different macro and micro elements were satisfactorily quantified in 30	Xiao et al. (2016)

Microgreens names	Microgreens properties	References
	distinct microgreen cultivars fall under to the Brassicaceae family with the use of ICP OES for mineral element detection.	
Three kind of broccoli Sicilian sprouting broccoli landrace (“*Broccolo Nero*”), the broccoli standard (“*Cavolo broccoli Ramoso Calabrese*”) and the standard commercial cultivar of kale (“*Cavolo Lacinato Nero di Toscana*”).	The ascorbic acid content varied greatly depending on the cultivar and stage of plant growth, with the microgreens of “Broccolo Nero” exhibiting the greatest value. “Broccolo Nero” (BN) and “Cavolo Broccolo Ramoso Calabrese” (CR) were found to have the highest polyphenol content; in general, sprouts displayed much higher values than microgreens and baby leaves.	Di Bella et al. (2020)
Five Brassicaceae species: broccoli (*Brassica oleracea* L.), daikon (*Raphanus raphanistrum* subsp. *sativus* (L.) Domin), mustard (*Brassica juncea* (L.) Czern.), rocket salad (*Eruca vesicaria* (L.) Cav.), and watercress (*Nasturtium officinale* R. Br.).	High levels of ascorbic acid observed in mustard. Broccoli had the highest levels of polyphenols, carotenoid pigments, and chlorophyll, along with a strong antioxidant capacity. High levels of total sugar were characteristics of mustard. To determine each species’ profile and isothiocyanates content compounds with numerous documented health advantages. The essential oil composition of each was discovered.	Marchioni et al. (2021)

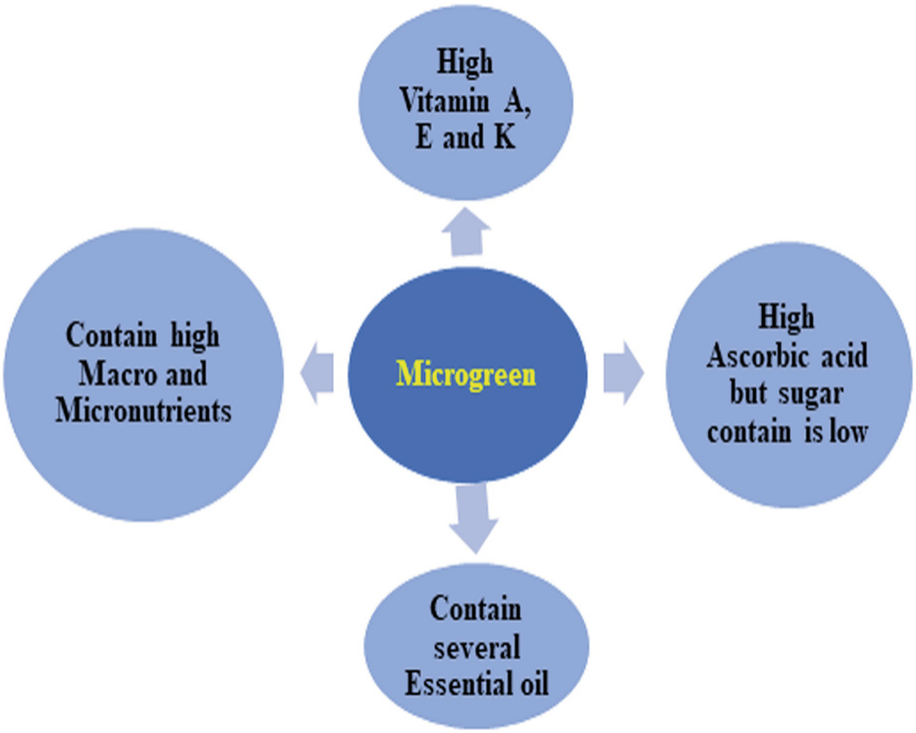


Fig. 2 Microgreens contain several essential oils, high Ascorbic acid, high macro-micronutrients and high vitamins A, E, and K

3.1 Vitamins

Humans need trace amounts of chemical molecules called vitamins. Every one of them helps differently to keep our bodies healthy. Vitamin is essential for body function and metabolism. The microgreen contains high amounts of vitamins like Vitamin E, A, K and C. Some of the vitamins which is present in higher concentrations in microgreen are:

3.1.1 Ascorbic Acid—Vitamin C

The body needs ascorbic acid, sometimes referred to as vitamin C, as a vital bioactive phytochemical in order to function effectively. It also falls within the category of antioxidants that support different human metabolisms (Bhaswant et al. 2023). The 25 microgreens' total ascorbic acid (TAA) contents varied from 20 to 147 mg/100 g FW. The samples with the greatest TAA levels were red cabbage

and garnet amaranth microgreens, followed by opal radish, China rose radish, and opal basil. Red cabbage microgreens had a vitamin C concentration of 147.0 mg/100 g FW, which was 2.6 times higher than that of mature red cabbage (57.0 mg/100 g FW) found in the USDA National Nutrient Database for Standard Reference and six times higher than previously reported data for the same crop (17.4 mg/100 g FW) (Xiao et al. 2012). Total vitamin C and K showed a substantial positive association ($p < 0.01$), while chlorophylls, P, N, S, Ca, B and Mg showed a negative correlation. Future studies on vitamin C biofortification using other microgreens can make use of these correlations. By adding extra ascorbic acid to a plant's diet raised its vitamin C content by 222% while maintaining the plant's mineral makeup (Kathi et al. 2023).

3.1.2 Phylloquinone—Vitamin K

Phylloquinone serves as vitamin K1, which is essential for bone growth, blood coagulation and vascular metabolism. Vitamin K1, which is primarily found in the photosynthetic parts of dark-green plants, is essential for blood coagulation. The vegetables broccoli (*Brassica oleracea* var. *italica*), kale (*Brassica oleracea* L. var. *acephala*), and spinach (*Spinacia oleracea* L.) are good sources of vitamin K. The 25 microgreens that were evaluated had phylloquinone concentrations that ranged widely, from 0.6 to 4.1 µg/g of fresh weight (FW). Among them, garnet amaranth (4.1 µg/g FW) had the highest concentration of phylloquinone, and the other phylloquinone concentrations are red cabbage (2.8 µg/g FW), pea tendrils (3.1 µg/g FW), green basil (3.2 µg/g FW), and red sorrel (3.3 µg/g FW) (Xiao et al. 2012).

3.1.3 α-Tocopherol—Vitamin E

Alpha-tocopherol is the most common and biologically active form of vitamin E. It prevents in vivo lipid oxidation and is a component of the cell's fat-soluble antioxidant system (Frankel 1989). The microgreens that contained the most α-tocopherol is radish (~59 mg/100 g). Microgreens from mustard, fennel and sunflower were also excellent providers of α-tocopherol. The microgreens fenugreek showed the lowest amount of α-tocopherol (5.0 mg/100 g) (Ghoora et al. 2020). Furthermore, peppercress, cilantro, and orange radish microgreens are also great providers of α- and γ-tocopherol, with α-tocopherol concentrations ranging from 12.5 to 16.7 mg/100 g FW and γ-tocopherol concentrations from 41.2 to 53.1 mg/100 g FW (Xiao et al. 2012).

3.1.4 β-Carotene—Pro Vitamin A

β-Carotene is a red-orange pigment that is prevalent in fruits of plants. It is an organic substance. The body converts beta-carotene to vitamin A (retinol). We need vitamin A for good vision, strong immunity, healthy skin, healthy mucous membranes, and healthy eyes. It was discovered that the microgreens were moderately to very well-sourced in vital elements, dietary fibre, and protein. Vitamin E, beta-carotene (pro-vitamin A) and Vitamin C were all abundant in them, fulfilling 28–332%, and 24–72%, 28–116% of the recommended daily intake of each vitamin, respectively. Regarding vitamin A, it was discovered that five microgreens—fennel (71.8%), radish (51.6%), mustard (58%), French basil (53.5%), and roselle (50%)—contributed >50% to the Nutrient composition (NC) (Ghoora et al. 2020).

3.2 Carotenoids

Tetraterpene pigments with the colours yellow, orange, red, and purple are also called carotenoids. Carotenoids are divided into two categories: carotenes and

xanthophylls. Green leafy vegetables and yellow-orange fruits are typically thought to be the richest sources of carotenoids. Carotenoids are important because they protect plants from photooxidative processes and protect people from cancers of the liver, colon, lung, pancreas, or prostate, among other organs. They are effective antioxidants as well. Research has shown that consuming goods high in carotenoid content can improve cardiovascular and degenerative disorders. The range of values in microgreens was 1510–4073 ~g/g FW. The following order of increase: radish, beetroot, green pea, kale, amaranth. Carotenoids contained in sprouts varied more widely, from 22 ~g/g FW for mung beans to 949 ~g/g FW for radish. The sample under analysis contained significant amounts of oxygenated carotenoids, including lutein, neo-, and zeo-xanthin, as well as α carotenes, and β carotene. Compared to sprouts, the microgreens had higher concentrations of (α+β)-carotene, lutein, neochrome, zeo- and neo-xanthin (Wojdyło et al. 2020).

3.3 Total Sugar Contents

Microgreens are poor in sugar and rich in organic acids, carotenoids, and chlorophylls. Many microgreens contain different kinds of sugar like glucose, fructose and sucrose. This sugar increases the taste of the microgreen and baby leaves. A detector for differential refractometer (Waters Corp., model number 410) was used to identify individual sugars. The total sugar content was given as the product of the glucose, fructose, and sucrose contents reported in grams per kilogram (Xiao et al. 2015).

3.4 Minerals

Microelements are those that the body requires in minute levels, such as Mn, Cu and Zn etc. whereas macro elements are those that the body requires in higher proportions, such as Ca, K, Mg etc. Many elements like Mg, Mn, Cu, and Zn

were more abundant in microgreens than in the vegetable. The amounts of Mn, Zn, P, Fe, K, Mg, Ca, Na and Cu were higher in the microgreens grown on compost (C) than in the vegetable. The average compost-grown microgreen: vegetable nutrient ratio was 1.73 for eight nutritionally significant minerals (Mg, Mn, P, K, Ca, Fe, Zn, and Na). However, hydroponically produced microgreens only exhibited average ratios of 0.86 (hydroponically grown) and 0.95 (Only water grown) for these minerals, suggesting that their nutritional value was marginally lower than that of the vegetable (Weber 2017). According to another study's findings, eating microgreens from the Brassicaceae family is a good way to meet the requirements of element dietary reference intakes, especially for children, while also providing a balanced diet by providing both macroelements (like K and Ca) and microelements (like Fe and Zn) (Xiao et al. 2016).

3.5 Polyphenols and Glucosinolates

Plant foods contain a group of chemicals called polyphenols, which include lignans, flavonoids, phenolic acids, and stilbenes. There were notable variations in the amounts of polyphenols present in the seeds of the three varieties of Brassica oleracea. According to the HPLC study, the primary polyphenols (PPH) components of the cultivars under investigation are represented by derivatives of gallic and sinapyl acids. Gallic acid derivative level ranged from 0.206 mg g^{-1} d.w. in Broccolo Nero (BN) to 0.525 mg g^{-1} d.w. in Cavolo Lacinato Nero di Toscana (CL), the sample with the greatest content. Cavolo Broccolo Ramoso Calabrese (CR) had the highest concentration of sinapyl acid derivatives (3.572 mg g^{-1} d.w.) whereas BN had the lowest concentration (2.826 mg g^{-1} d.w.) (Di Bella et al. 2020).

3.6 Chlorophyll Pigment Content

The green pigment called chlorophyll is found in the chloroplasts. Chlorophyll is an essential component of plants for photosynthesis. Microgreens treated with lower concentrations of Ascorbic acid (i.e., 0.05% and 0.1%) in trial 1 and 0.25% Ascorbic acid treatment in trial 2 exhibited higher values of chlorophyll a & b, TC, and carotenoids (Kathi et al. 2023). The chlorophyll content of microgreens varied from 196 to 638 ~g/g FW, with the decrease in order kale amaranth > green peas > beetroot > radish exhibiting the lowest chlorophyll level. The range of values for the chlorophyll content in sprouts was ~6–108, with lentils having the highest value (Wojdyło et al. 2020).

3.7 Essential Oil Content

All plant organs can acquire essential oils, however in different proportions. As a result, we may encounter them in the bark, stem wood, roots, leaves, flowers, and fruits. For the red variety (crispa), D-limonene was the principal volatile released by whole leaves; for the green variety, it was 2-hexanoylfuran. Furthermore, adult leaves have higher antioxidant potential and total phenolic content (TPC). These findings clearly show that the distinct scent of Perilla microgreens varies depending on the variety and is unrelated to the leaves' total phenol content or antioxidant level (Dimita et al. 2022). Over 97% of the contents of broccoli's essential oil were isothiocyanates, with 4-pentenyl, phenethyl, and 3-butenyl isothiocyanates accounting for more than 95% of the total. The oil of Daikon's made up of non-terpene derivatives chemical (~27%), (Z)-3-hexen-1-ol (~11%) and nonanal (~5.3%). Monoterpene hydrocarbons, primarily myrcene (83.7%) and limonene (7.5%), constituted the majority of the essential oil in rocket salad. Only caryophyllene (4.4%) and

humulene (1.3%), which belong to the sesquiterpene hydrocarbons group, were present in the group (Marchioni et al. 2021).

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4 Health-Promoting Effects

People all around the world are currently dealing with a variety of diseases. People's unhealthy eating habits are the primary cause of all of these diseases. Therefore, people looking for high-quality food sources in their diets. Microgreens, which are high in vitamins and minerals, can be used as a food of this quality. Microgreens, which are the sprouted parts of vegetables, herbs and greens, are rapidly gaining popularity for their nutritional qualities. They have higher levels of antioxidants, vitamins and minerals than fully-grown veggies. Microgreens provide up to 40 times higher levels of vitamin C, beta-carotene, and other nutrients than mature plants. They are also rich in bioactive compounds like phenolics and glucosinolates, which may play a role in different types of chronic diseases (Rizvi et al. 2023). In the past 10 years, the therapeutic benefits such as anti-oxidant, anti-diabetic, anti-inflammatory, anti-carcinogenic and many other activities, have been extensively examined in a variety of in vitro and in vivo investigations, as well as relevantly administered in clinical studies (Le et al. 2020; Partap et al. 2023) (Table 2). Lettuce microgreens are rich in bioactive compounds like carotenoids, anthocyanins, ascorbic acid, phenols and other bioactive compounds, which are highly effective against various diseases and boost immunity power (Martinez-Ispizua et al. 2022) (Table 2). Microgreens are a flexible item that can be easily cultivated in a very short time, so they can be easily added to our diet.

Table 2 Effective compounds in microgreens and their activities

Activities	Microgreens	Effective compounds	Reference
Anti-oxidant potential	Members of Brassicaceae, Amaranthus, poi, pumpkin, water spinach, palak, jute, cucumber, bottle gourd etc.	Carotenoides, tocopherols, ascorbic acid, glucosiolates & phenolic compounds, DPPH etc.	Xiao et al. (2019); Yadav et al. (2019)
Cardiovascular diseases	Red cabbage	Polyphenols and glucosinolates	Partap et al. (2023)
Anti-diabetic and anti-obesity	Millet, broccoli	α-Glucosidase and α-amylase (anti-diabetic); glucosinolates, glucoraphanin fiber, and vitamin C (anti-obesity)	Li et al. (2021); Bala Durairajan et al. (2024)
Anti-cancerous	Broccoli, cabbage, kale, lettuce, cucumber	Sulforaphane (SFN), lutein, zeaxanthin etc.	Bi et al. (2013); Liang et al. (2019)
Anti-inflammatory	Broccoli, cabbage, species of Fabaceae, red cabbage, kohlrabi, and purple radish	Phenolic compounds, vitamin-A, C & E, isothiocyanates and zeaxanthin	Zhang et al. (2021); Bhaswant et al. (2023)
Chronic kidney disorder	Chicory, lettuce, radish	Low potassium content, glucosidase-inhibiting components	Renna et al. (2018); Mohamed et al. (2023)
Iron deficiency	Brassicaceae microgreens, fenugreek, lettuce, broccoli	Consist of higher amount of iron	Sarwar et al. (2020); Khoja et al. (2020); Ghoora et al. (2020)
Biofortification of essential nutrients	Arugula, Brassicaceae	Vitamin-C, calcium, potassium, sodium, ferum & zinc	Di Gioia et al. (2019); Mezeyova et al. (2022)

4.1 Anti-oxidant Potential

Anti-oxidant potential is the ability of a substance to neutralise free radicals. Free radicals are unstable molecules that can damage cells and are linked to various diseases, such as cancer, heart disease, and Alzheimer's disease (Partap et al. 2023). Microgreens are high in antioxidants and have a robust supply of minerals. Anti-oxidants can protect against cellular damage and help lower the chance of contracting specific illnesses. Hydroponic Brassicaceae (microgreen) contains polyphenols and glucosinolates; when they break down, they become anti-oxidants. They can help reduce inflammation and prevent the growth of cancer cells (Xiao et al. 2019). Some summer microgreens, like cucumber, amaranthus, radish, spinach, etc., contain flavonoids and phenolic compounds. Additionally, these veggies exhibit DPPH (2,2-diphenyl-1-picryllhydrazyl), CUPRAC (cupric-reducing antioxidant power) and FRAP (ferric-reducing antioxidant power) in vitro assays. Therefore, they show anti-oxidant capacity (Yadav et al. 2019) (Fig. 3).

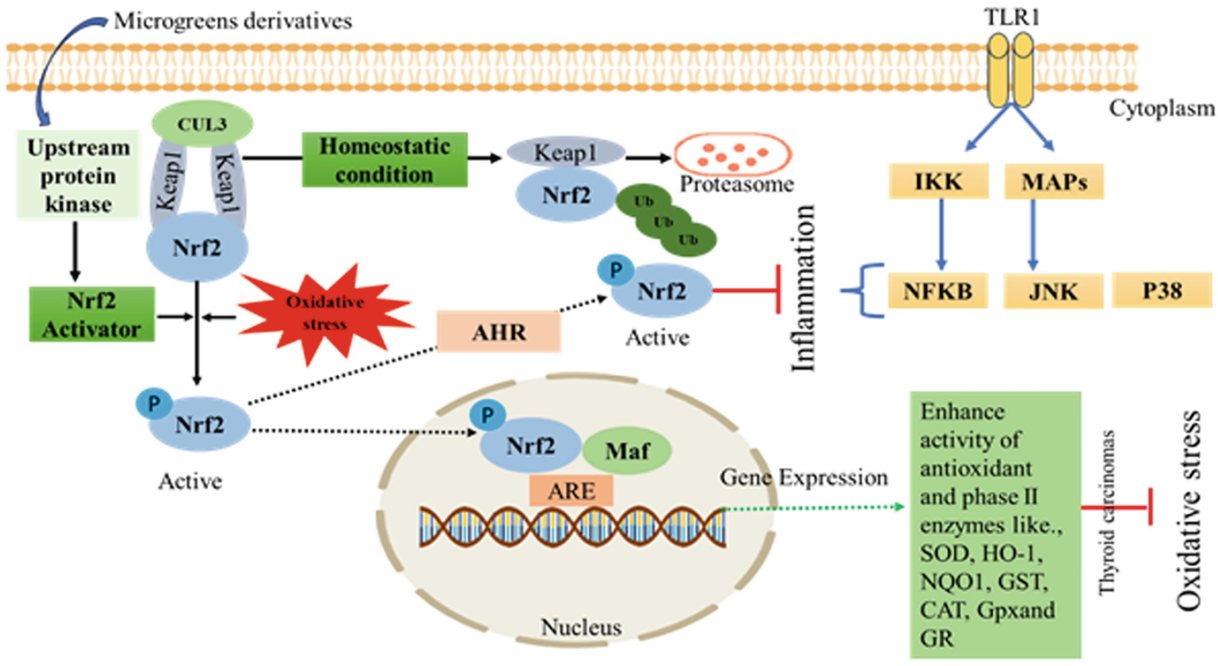


Fig. 3 Antioxidant and anti-inflammatory effects of polyphenols in relation to Nrf2 pathway with some modification (Lee et al. 2017; Heydarzadeh et al.

2022)

4.2 Cardiovascular Disease

Cardiovascular disease is a very common disease in the present day. It occurs due to a bad or poor diet. If we use microgreen vegetables in our diet, then we can prevent this disease issue. Because microgreen vegetables have anti-oxidant properties that prevent cardiovascular diseases, Microgreens regulate adipose tissue and control body weight; therefore, they are effective against cardiovascular disease (Zhang et al. 2021). Cholesterol levels and lipid levels are controlled by red cabbage, which is produced from microgreens (Partap et al. 2023). Microgreens can help raise good cholesterol and decrease bad cholesterol, according to certain research. This may be due to the presence of fibre, which helps inhibit cholesterol absorption (Ebert 2022). One of the main risk factors for cardiovascular disease is inflammation. Microgreens can help reduce levels of inflammatory compounds (Bhaswant et al. 2023).

4.3 Anti-diabetic and Anti-obesity Activity

Diabetes is a chronic condition caused by high blood sugar levels. According to Zhang et al. (2021), microgreens control blood sugar. Microgreen from millet consists of α-glucosidase and α-amylase that reduce sugar uptake by the blood, and microgreen from millet also shows anti-obesity activity (Bala Durairajan et al. 2024). Broccoli microgreen juice is very effective on adipose tissue, as it reduces white adipose tissue mass in the liver of mice (Li et al. 2021). So, broccoli microgreen juice can be kept in the diet as an anti-obesity food.

4.4 Anti-Cancerous Activity

Cancer is a very dreaded disease worldwide. Cancer treatment is also a very complex process. But if we include

some fresh microgreens in our daily diet, then we can stay away from cancer. Sulforaphane is an anti-cancer agent. Some cruciferous microgreens, such as broccoli, cabbage, and kale, are rich in sulforaphane (SFN). SFN can block the growth and spread of cancer cells (Liang et al. 2019). Lettuce, kale, and cucumber can also be used as microgreens; they contain some anticancer chemicals like lutein and zeaxanthin. Zeaxanthin and lutein protect cells from the damaging effects of free radicals (Bi et al. 2013). Thus, microgreens are an excellent supplement to a balanced diet that can help reduce the risk of cancer.

4.5 Anti-Inflammatory Activity

Microgreens are known for their nutritional value, but they also have anti-inflammatory properties that may benefit various health conditions. According to numerous studies, microgreens have a high antioxidant content and many vitamins (vitamins—A, C & E), which save cells from damage caused by free radicals. Free radicals are associated with inflammation (Bhaswant et al. 2023). Microgreens contain minerals such as magnesium and potassium that can aid in muscle relaxation and blood pressure regulation, which can help reduce inflammation. Microgreens contain a variety of phytochemicals, including isothiocyanates and zeaxanthin; they exhibit anti-inflammatory properties (Zhang et al. 2021) (Fig. 3).

4.6 Chronic Kidney Disorder

Microgreens may be a helpful addition to the diet for people with chronic kidney disease because they can be grown with a low potassium content. Potassium is important for healthy blood pressure, but patients with chronic kidney disorders often have low potassium intake because their kidneys cannot remove excess potassium from the blood. Studies have shown that microgreens grown in low potassium environments have significantly

low potassium levels, and they maintain their nutritional value and antioxidant activity (Renna et al. 2018). Another study also revealed that radish microgreens prevent kidney disorders due to toxicity (Mohamed et al. 2023).

4.7 Iron Deficiency

Iron, a mineral required for the production of red blood cells, which transport oxygen throughout the body, is abundant in microgreens. Anaemia from an iron deficit can result exhaustion, weakness and dyspnea. Most of the microgreen plants in Brassicaceae contain a higher amount of iron and other micronutrients (Ghoora et al. 2020). Studies have shown that fenugreek microgreens have particularly high iron levels (Sarwar et al. 2020). Immature lettuce and broccoli are very rich in iron (Khoja et al. 2020).

4.8 Biofortification of Essential Nutrients

Microgreens hold immense potential in biofortification, a strategy to increase the concentration of essential nutrients in crops. Due to microgreens' rapid growth cycle, they concentrate certain nutrients more effectively compared to mature plants. Studies revealed that microgreens can contain significantly higher levels of vitamins, minerals, and antioxidants than their full-grown counterparts (Mezeyova et al. 2022). Biofortification of arugula microgreens is rich in vitamin C, Ca, K, Na, etc. (Kathi et al. 2024). Agronomic biofortification of microgreens is able to meet the needs of Fe and Zn as essential nutrients (Di Gioia et al. 2019).

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5 Future Research Perspectives

As conferred in this chapter, the functional role of microgreens has been summarised from different scientific literature. As the study of microgreens is still in early

phases, so there is an opportunity or plentiful room for ongoing researchers, and maybe countless information gaps remain to be occupied by continuous scientific communication. Holistic multi-nutritional properties and chemical compounds evaluation discussed in this chapter, where the dietary value of different microgreens needs to be assessed in future using holistic research approaches instead of concentrating on nutritional properties.

Moreover, as discussed in this chapter, the overall nutritional benefits of some specific microgreens. Also, different articles have documented the nutritional properties of microgreens, but the comparison of nutritional profiling of the same species in between the microgreens and mature plant species are still needed in modern sciences.

However, some precise modes of action of microgreens are elaborated in this chapter, but the proper way of route to inducing the health-promoting benefits is still unclear and unknown to the researcher. It is well known how microgreens participate in different pathways of cellular processes to overcome the concerned human disease, but the activity of microgreens to prevent specific chronic illnesses by proper molecular mechanisms is required by thorough research in higher model organisms.

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6 Conclusion

Microgreens, a newly plant-based multifunctional nutritious food and good sources of bioactive compounds, play great potentiality towards human health promotion by daily consumption, according to the published article. Microgreens (edible plants) are stellar sources of different bioactive compounds and phytochemicals, such as various essential minerals, anthocyanins, carotenoids, chlorophyll, glucosinolates, polyphenols, etc., which significantly impart with high anti-inflammatory, antioxidant and anti-diabetic

effects. So, due to the varied functional properties of microgreens, it can be considered as a sustainable alternative food for human health benefits.

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Vigna radiata: a Minor Legume with Major Potential

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Abbreviations

ACIAR Australian Center for International Agricultural Research
AP Apetella
APX Ascorbate peroxidase
BARI Bangladesh Agricultural Research Institute
bHLH Beta-helix loop helix

CAT Catalase
ChlF Chlorophyll fluorescence
CLS *Cercospora* leaf spot
cm Centimetre
DAR Department of Agricultural Research;
DNA Deoxyribonucleic acid
EREBP Ethylene-responsive element binding protein
Fig Figure
gm Gram
GSH Glutathione
GWAS Genome-wide association study
h Hours
H_2O_2 Hydrogen Peroxide
HSPs Heat-shock proteins
ICAR Indian Council for Agricultural Research
IIPR Indian Institute of Pulses Research
IMIN International Mungbean Improvement Network
Kcalories Kilocalories
kg Kilogram
MAS Marker-assisted selection
max Maximum
Mb Megabyte
mg Milligram
min Minimum
mM Millimolar
mM/L millimoles per litre
mmhos/cm Millimhos per centimeter
MYB Myeloblastosis viral oncogene homolog
MYMD Mungbean yellow mosaic disease
NGS Next-generation sequencing
PA Phytic Acid
PEG Polyethylene glycol
QTL Quantitative trait loci
RFLP Restriction Fragment Length Polymorphism
RNA Ribonucleic acid
ROS Reactive oxygen species
rRNAs Ribosomal RNAs

SDGs Sustainable development goals
snoRNAs Small nucleolar RNAs
SNP Single nucleotide polymorphism
snRNA Small nuclear RNAs
SOD Superoxide dismutase
SSR Simple sequence repeats
ton/ha tonnes per hectare
tRNAs Transfer RNAs
ULCV Urdbean leaf crinkle virus
USA United States of America
USD United States dollars
USDA United State Department of Agriculture.
°C Degree Celsius

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1 Introduction

Food is a prime aspect of life and has been put under basic human rights by many governments across the globe. The United Nations also has put “a world without hunger” in its first sustainable development goals (SDGs). However, meeting this goal of “a world without hunger” is a daunting task and requires support and activities from multiple spheres of society, nevertheless, the primary role is to be played by agriculture and related activities. As per an estimate, in the last half-decade, as much as 71% of the households globally endured food insecurity in one or other form. This insecurity time interval ranged from 1 month to as long as 6 months in countries like Ethiopia (Niles and Salerno 2018; FAO 2009). It is expected that by 2050, an increase of 70% in agricultural production will be required to meet the global food requirement. The two important factors that hamper agricultural production are biotic and abiotic stress factors that are further aggravated by global climate change and limited diversity in crop selection. Overall, the role of global climate change in agriculture underscores the need for proactive measures to build resilience, enhance adaptive capacity, and promote sustainable food production systems. Though ways and strategies are being planned and executed to tackle global climate change, it remains out of the purview of this current chapter. The very limited diversity in crop selection is a matter of concern today. The global food supply relies heavily on a small number of crops, with four main crops—rice, wheat, maize, and potatoes—providing the majority of daily caloric intake. However, minor crops play a crucial role in local and regional food systems, contributing to dietary diversity and resilience (Hilger et al. 2015). Minor crops, including staples,

are essential for ensuring food supply during specific periods and promoting a nutritionally balanced diet, not to mention these crops are well suited to be cultivated on marginal lands and can provide valuable genetic resources for crop improvement as well. Minor crops, including staples, are essential for ensuring food supply during specific periods and promoting a nutritionally balanced diet. They are often well-adapted to marginal conditions and can provide valuable genetic resources for crop improvement as well. Biodiversity in agriculture, including minor crops and wild relatives of major crops, helps farmers manage risks associated with pests, diseases, natural disasters, and soil degradation. Diverse production systems are better equipped to adapt to environmental variations and attenuate the impacts of crop loss, particularly at the onset of global climate change, promoting biodiversity in agriculture, supporting the cultivation of minor crops, and empowering smallholder farmers with sustainable technologies are crucial steps in ensuring food security, resilience, and sustainability in the face of current global challenges (Tutwiler 2014; FAO 2017; Bacon et al. 2014; Pataczek et al. 2018).

Mungbean (*Vigna radiata* L. Wilczek) is one such minor legume crop that provides a valuable opportunity for dryland smallholder farmers to enhance the sustainability and profitability of their farms. The cultivation of mungbean presents an opportunity for smallholder farmers to snap the cycle of dipping productivity, especially in the arid and semi-arid regions, increase farm profitability, and enhance agricultural sustainability through diversified cropping systems. It is a nutritious warm-season legume crop, with grains rich in protein, minerals, and vitamins. Its cultivation can contribute to improving the dietary diversity and nutritional status of communities where it is grown.

The origin and domestication of mungbeans have been traced back to 1500 BC from the Indian subcontinent. This region served as the cradle of agriculture for many crops, including mungbean. From its origins in the Indian subcontinent, cultivated mungbeans were gradually introduced to southern and eastern Asia. These regions provided favourable climates and agricultural conditions for the cultivation of mungbean, leading to their widespread adoption as a staple food crop. Over time, mungbeans were introduced to Africa, where they became an important component of local diets and agricultural systems. The adaptability of mungbean to diverse climates and soil conditions allowed them to thrive in various regions of the continent. Today, cultivated mungbeans are widespread throughout the tropics, ranging from sea level to altitudes of up to 1850 meters in the Himalayas. Their adaptability, nutritional value, and versatility have contributed to their popularity as a food crop in diverse cultural and geographic contexts (Lambrides and Godwin 2007; Mogotsi 2006). It is cultivated widely across Asia, parts of Africa, and Australia. About 90% of the mungbean is produced from Asia and India stands as the foremost producer of mungbean accounting for over 50% of the global production. After India, China ranks to be the second-largest producer of mungbean globally, dedicating 19% of its legume production to mungbean. Furthermore, Thailand serves as the primary exporter of mungbean, witnessing a notable 22% annual increase in production between 1980 and 2000 (Lambrides and Godwin 2007).

Its adaptability to diverse agro-climatic conditions makes it suitable for cultivation in varied regions, particularly in dryland areas with limited water availability. To enhance the sustainability of agricultural systems in semi-arid and arid regions, mungbean could be integrated into the cropping cycle, particularly in South and Central Asia. Mungbean could be valuable in providing additional income to farmers by serving as a valuable catch crop, in addition to improving soil fertility through nitrogen fixation (Lambrides and Godwin 2007; Pataczek et al. 2018). Diversification of local production systems by including mungbean can provide smallholder farmers with additional sources of income. Its relatively short growing cycle allows farmers to intercrop or rotate it with other crops, maximizing land productivity and economic returns.

Despite being nutrient-rich and also contributing to food security mungbean faces many obstacles at its various stages of growth ranging from seedling to maturation stage making it difficult to be adopted as one of the mainstream crops. Global warming and rapid climate change characterized by extreme temperature conditions (extreme high and extreme low temperature) and frequently occurring floods and droughts are the major abiotic stressors that affect mungbean (Pratap et al. 2019). Besides climatic factors, poor irrigation practices result in increased occurrence of pests and diseases as well as deteriorated soil condition which includes increased salt content. These abiotic and biotic stressors have resulted in decreased productivity and have also negatively affected the cellular metabolism, growth and yield potential of the crop plant (Pandey et al. 2018), piling on to the reluctance in the acceptance of mungbean in the main cropping system. It is crucial to address these issues through breeding and scientific study to maintain mungbean production and increase its resistance to various stressors. In recent years, advancements in molecular, genomic, and proteomic tools have provided new insight for studying and improving mungbean. The interpretation of the genetics of important traits, such as stress tolerance or response to different environmental stressors, yield potential, and nutritional quality, can help scientists facilitate the development of improved cultivars with enhanced agronomic performance and nutritional value that can meet the growing demands of the rapidly increasing population.

2 Agronomic Properties

Mungbean is a highly branched annual crop with a tentative height of about 60–80 cm. The central stem and side branches are erect and semi-erect respectively and the leaves are trifoliate. Flowers are present in clusters of about 12–15. The flowers later develop into small cylindrical pods. The pods are primarily linear, however, sometimes they could also be round, slender or curved and contain small almost globular seeds (Sefa-Dedeh and Stanley 1979; Oplinger et al. 1990). It is a self-pollinating and short-duration crop plant, usually maturing within a span of 60–90 days (Kumar and Kumar 2014).

There are majorly two types of mungbean cultivars, distinguished based on the seed color. One of them is the golden gram, characterized by its yellow

seeds, lower seed yield, and a tendency for pods to shatter at maturity. These are often grown for forage or green manure. The second cultivar is green gram characterized by bright green seeds, higher productivity, more uniform ripening, and less susceptibility to pod shattering (Swaminathan et al. 2012). Apart from these, two additional types of mungbean cultivar are recognized in India, having brown and black seeds (Mogotsi 2006). The mungbean roots also exhibit symbiotic association with *Rhizobium* sp. and *Bradyrhizobium* sp. which aid in nitrogen fixation thereby increasing soil fertility and crop productivity (Allito et al. 2015; Fuskhah et al. 2019; Yimram et al. 2009).

3 Nutrient Value

As mungbean is a nutrient-rich food source, packed with various vitamins (niacin, thiamine, riboflavin, vitamin K and vitamin C), essential minerals (calcium, iron, potassium, zinc, magnesium and manganese) with 24–28% protein content, it can be incorporated into diets in various forms, including whole beans, as sprouts, flour or as paste (Itoh et al. 2006). Besides 24% protein content, it also contains 59–65% carbohydrate, 1–1.5% fat, 3.5–4.5% crude fiber, 4.5–5.5% ash, 0.0073% iron, 0.326% phosphorus, 0.00039% carotene, and 0.0021% niacin on dry weight basis. Additionally, 100 gm of mungbean is estimated to provide an energy value of 334–344 kcalories (Swaminathan et al. 2012; Adsule et al. 1986). The presence of secondary metabolites like flavonoids and phenols in various parts of mungbean which includes seeds, seed coats, and sprouts has attracted growing attention due to their health-promoting benefits (Ganesan and Xu 2018). Amongst secondary metabolites phytic acid (441.5 mg/100 gm), poly-phenols (462.5 mg/100 gm), tannins (366.6 mg/100 mg), proteinase inhibitors and hemagglutinin are the major ones. The nutrient distribution is not uniform across the various parts of seed. Embryos contain the highest amount of protein and lipids; seed coats and cotyledons are rich in crude fiber and starch respectively (Mubarak 2005). Apart from being nutrient-rich the plant also exhibits a range of morphological and physiological characteristics that contribute to its resilience and productivity (Sehrawat et al. 2019).

4 Genetics of *Vigna radiata*

V. radiata possesses a comparably compact genome of 579 Mb, having a chromosome number of 2n = 2x = 22. (Parida et al. 1990; Kang et al. 2014). The genome information revealed that about 50.1% was occupied by repetitive sequences and among them, long terminal repeat (LTR) retrotransposons make up the largest portion with 36.5% of the total *V. radiata* genome. In the genome in total 22,427 protein-coding genes were identified. 2310 non-coding genes were also identified which included 629 tRNAs, 537 microRNAs, 717 snoRNAs, 280 rRNAs, 110 snRNA and 37 other regulatory RNA genes. Further, 1850 genes encoding for various transcription factors were also identified. The most abundant transcription factor genes were MYB class

followed by Apetella (AP) 2 / ethylene-responsive element binding protein (EREBP) and beta-helix loop helix (bHLH) classes. The *V. radiata* genome retained the basic genome architectural plan of most of the other model plant species such as *Arabidopsis thaliana*, *Oryza sativa* however, one notable difference was the very minimal presence of bZIP2 family of transcription factors. It was found to be less than 1% of total transcription factors present in *V. radiata* while the same was found to be over 3% in the rest of the legumes (Kang et al. 2014).

The trait of plant height is controlled by a single dominant gene in *V. radiata* (Talukdar and Talukdar 2003). The purple hypocotyl colour is dominant over the green hypocotyl colour and is controlled by a single dominant gene (Bose 1939). The large size of the leaflet is dominant over the small leaflet and is controlled by a single dominant gene (Dwivedi and Singh 1985). The two dominant genes namely I1 and I2 control the simple inflorescence while the homozygous double recessive form leads to the compound inflorescence (Sen and Ghosh 1959). The dominant form of gene C controls the presence of one cluster per node while the recessive form leads to three clusters per node (Singh and Singh 1995). The light yellow olive colour of flowers is dominant over the olive yellow colour and the same gene also drives the colour of the unripe pod (Murty and Patel 1972; Bose 1939). The shattering type of pod is dominant over the non-shattering type (Singh et al. 1975). The albino mutants of *V. radiata* are also available (Bahl and Gupta 1984). The insensitiveness of the photoperiod is dominant over the photoperiod sensitivity (Verma 1971).

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5 The International Cooperation in Mungbean Research: An Update

In spite of being an ideal candidate for genomics and molecular research, the breeding and sequencing studies in *V. radiata* were always underrated and underfunded both at national and international levels. It is worthwhile to mention here that mungbean was the first legume in which genomic research was started however, later it took a back step and currently it is lagging behind most of the other legumes (Somta et al. 2022). The first RFLP and SSR markers for the mungbean were identified way back in 1992 followed by quantitative trait loci (QTL) mapping for *Cercospora* leaf spot (CLS) and powdery mildew. The simple sequence repeat (SSR) marker was further enriched in 2002. The next-generation sequencing (NGS) techniques were applied to mungbean in the late 2000s. NGS-based SSR markers were identified in 2009 and enriched in 2012. NGS-based single nucleotide polymorphism (SNP) identification was done in 2010. A genome-wide association study (GWAS) was reported in 2018. The first draft genome sequence of mungbean was reported in 2014 and an improved genome sequence was reported in 2021 (reviewed in Somta et al. 2022).

Before the advent of the molecular genomics era, breeding was the gold standard for the development of newer and better plant varieties. The earliest

mungbean research and breeding started in India, the Philippines and the USA way back in the 1920s and it started with the isolation of pure lines (Bose 1932). The first commercial variety in India was released in 1936 and was named Type 1. In 1948, the first commercial variety developed by hybridization techniques was released and was named Type 44. So far, all the cultivars released had small seeds, non-uniform maturity, low yield and photoperiod sensitivity and the average yield was ca. 0.4–0.8 ton/ha. Later, the breeding for large seeds, uniform maturity, photoperiod insensitivity and disease resistance started. In the Philippines, the collection of various landraces and germplasm started way back in 1916 and the breeding program started in 1933 (Catipon et al. 1988). In the year 1956, a national breeding program was set up in the Philippines with the aim of developing high-yielding, uniform maturity, large-seeded, non-shattering, high protein and carbohydrate content and semi-dwarf variety. This led to the development of two commercial cultivars MG50-10A and MD15-2 in the year 1969 with a yield potential of 1.2 tons/ha (Ballon et al. 1977; Catipon et al. 1988). Later, in mid-1970s the breeding for disease resistance and good plant architecture started with the germplasm obtained from India (Catipon et al. 1988). The United State Department of Agriculture (USDA) started the mungbean program way back in 1914 with the help of germplasm obtained from various other countries and three cultivars namely Berken, Oklahoma and Kiloga were released for commercial use in the year 1963 (Matlock and Oswalt 1963). With the establishment of the World Vegetable Center (WorldVeg, formally known as the Asian Vegetable Research and Development Center), in Taiwan in the year 1971, the international cooperation in mungbean breeding gained momentum. Since its inception, it has played a crucial part in the collection of mungbean germplasm followed by its characterization and selective breeding. It has used the high-yielding and superior agronomic attributes of improved cultivars in the breeding programs and has led to the development of superior varieties with yield potential up to 1.8–2.2 tons/ha (Fernandez and Shanmugasundaram 1988). Scientists in several countries have used these varieties as parents for breeding programs. As per an estimate by Sequeros et al. (2021) the varieties released by WorldVeg led to an economic gain to the tune of 1.4 million United States dollars (USD) in Myanmar from 1980 to 2016.

Though modern sequencing technologies have paved the way for mungbean molecular research, but it still lags with other crop species. In the future the foremost requirement would be to construct a high-quality reference genome, identifying the QTLs and genes controlling the major agronomic traits such as disease resistance, environmental stress resistance, yield etc. Recently, the International Mungbean Improvement Network (IMIN) has been constructed with the prime aim of strengthening mungbean research leading to the development of better and contemporary varieties.

6 Effect of Abiotic Stresses on Mungbean

Abiotic stress refers to the adverse impact of external environmental conditions that substantially affect the growth, development, and productivity of plants. Stressors such as extreme temperatures, water scarcity, and soil salinity trigger various responses in plants, including alterations in physiological, chemical, and morphological characteristics, cellular metabolism, as well as changes in growth rates and gene expression. These responses ultimately lead to reduced crop yields on a global scale (He et al. 2018). The nutritional, agronomic and economic benefits of mungbean are increasingly recognized worldwide, leading to a substantial increase in production and consumer demand over the past two decades (Kim et al. 2015). Mungbeans are presently produced on more than six million hectares (ha) of land worldwide, and they are cultivated in a variety of latitudes and seasons (Chauhan and Williams 2018). In spite of increased research and developmental strategies, mungbean production is unlikely to expand much further because of its low average yield and inconsistent productivity (Gayacharan et al. 2020). Currently, global mungbean production ranges from less than 1 to 1.5 tons per hectare which is significantly lower primarily due to abiotic stress conditions such as drought, and extreme temperatures occurring due to the escalating impact of climate change, with increasing climatic variability and frequency of extreme weather events. Abiotic stress conditions like drought and heat can significantly impair the morphology, physiology, and reproductive capacity of mungbean, ultimately affecting yield and grain quality (Sequeros et al. 2021; Jägermeyr et al. 2021). Secondly, due to poor irrigation practices, abiotic stress such as salinity also harms crop growth and productivity through biochemical and physiological changes (Jägermeyr et al. 2021). Therefore, urgent attention needs to be drawn to enhance abiotic stress tolerance and improve the yield stability in mungbean. Here, we specifically address the major abiotic stresses such as salinity, dehydration, high and low temperature stresses that impact mungbean during the vegetative and reproductive stage of their growth cycle. By deepening our knowledge of how stress influences growth and identifying adaptive mechanisms, the effectiveness and efficiency of selection in breeding programs can be enhanced which will further facilitate the development of mungbean varieties having enhanced climate resilience.

6.1 Heat Stress

Legumes like mungbean are generally cultivated in warm and humid climates and therefore are particularly susceptible only to very high temperatures (Bhardwaj et al. 2023). Mungbean cultivation during the summer season often encounters severe heat waves, with temperatures rising to 45 °C, research shows that in mungbean, temperatures exceeding 40 °C during the reproductive stage lead to terminal heat stress, resulting in impaired anthesis, reduced viability of pollen, decreased flower fertilization, enhanced flower drop, and shortened grain filling period (Basu et al. 2019). Even slight temperature increases can disrupt the crop cycle, accelerating flower drop, embryo abortion, and impeding grain filling (Hanumantharao et al. 2016). Patriyawaty et al. (2018) demonstrated that exposure of mungbean crops to

45 °C for 10 days during flowering significantly reduced the viability of pollen by 60%. This reduction in viability was likely attributed to decreased pollen size and the distortion of pollen grains. Flower shedding is prevalent during heat stress, with reported rates reaching up to 79% under heat-stress conditions (Kumari and Varma 1983). To cope with higher temperatures, plants utilize an important defense mechanism of dehydrating the pollen grains and embryos, which helps to enhance stress tolerance by maintaining viability under stressful conditions. This prolonged dormancy in embryos and reduced water content in pollen contribute to heat tolerance, although the effectiveness of these mechanisms varies across genotypes. Therefore, traits associated with pollen and embryo tolerance to heat stress are crucial for the development of heat-tolerant/resistant varieties of mungbean (Rainey and Griffiths 2005).

Not much information is available about the mechanisms behind high temperature-driven floral and pod growth in mungbeans. Khattak et al. (2009) reported that high temperatures did not reduce resistance to blossom shedding, however, this association might not hold true for every genotype (Aggarwal and Poehlman 1977). Temperature-flowering interactions have been documented by Rawson and Craven (1979) in specific genotypes with high mean temperatures and extended photoperiods were found to be 24–28 °C and 15–16 h respectively. Stress caused by heat is one of the main causes of failure in reproductive growth and seed production.

The primary morphological changes in a plant due to high-temperature stress leading to lowering of crop yield include discoloration of the leaves and stem, reduction in root and shoot length, falling of leaves, seed shrinkage, and damage to fruits (Vollenweider and Günthardt-Goerg 2006). Similar morphological changes were observed in two mungbean genotypes (SML 832 and SML 668) that were subjected to high-temperature stress treatment. As a response to the heat stress, it was observed that mungbean plants showed a fall in plant biomass (16–19%), a decrease in the total number of pod sets, and hence lower yield of seeds (35–40%), decreased number of filled pods (32–38%), and seed number (43–47%) (Kaur et al. 2015).

Elevated temperatures cause the leaves to become scorched, which can cause a decrease in leaf pigments and hamper the photosynthetic processes (Priya et al. 2019; Awasthi et al. 2015; Kaushal et al. 2013). Leaves play a crucial role in supplying sucrose and other vital macromolecules to reproductive components like flowers, pods, and seeds, as well as supporting nodulation and organelle function (Awasthi et al. 2015). Maintaining the photosynthetic machinery in leaves is essential for sucrose synthesis and transport. However, this process is disrupted due to heat stress.

The differences in response to heat stress among the mungbean varieties suggest variability in their heat tolerance mechanisms. The heat-sensitive genotypes (Basanti and MH 318) experienced greater losses in chlorophyll, carotenoids, and chlorophyll stability index, indicating their susceptibility to heat-induced damage. In contrast, the tolerant genotype (MH 421) exhibited maintained higher yield and physiological functioning under heat stress,

suggesting the presence of adaptive traits that enable it to withstand high temperatures more effectively (Chand et al. 2018).

Elevated temperatures exert a remarkable effect on the biochemical characteristics of mungbean plants. Higher temperatures particularly those exceeding 40°/30 °C (max/min) decrease the water potential of the leaf and elevate the oxidative stress levels. Oxidative stress occurs as a result of excess production and accumulation of reactive oxygen species (ROS) (Demidchik 2015). The rise in ROS such as hydroxyl radical, singlet oxygen, superoxide radical, and hydrogen peroxide leads to cytoskeleton damage and disrupts the composition of proteins, lipids, and DNA. These changes in cellular components under stress conditions result in enzyme inactivation and interfere with the essential metabolic processes (Bhardwaj et al. 2023). Exposure to high-temperature stress for an extended period can lead to cellular damage and can also result in cell death (Wahid et al. 2007). When plants experience heat shock, the activity of antioxidant enzymes such as catalase, superoxide dismutase and glutathione (GSH) declines, weakening the plant's defense system as well as leading to an oxidative burst (Rajput et al. 2021). According to Bita et al. (Bita and Gerats 2013), this disturbance may have a negative influence on cellular metabolism and lead to the build-up of toxic substances such as H_2O_2 and malondialdehyde, which can eventually decrease plant productivity. Elevated temperatures lead to decreased synthesis of carbohydrates and storage protein causing the shrivelling of legume seeds. The enzyme sucrose synthase is particularly important for grain filling, however, prolonged exposure to high temperatures can disrupt the enzyme activities, leading to inadequate grain filling (Basu et al. 2019). Heat stress also affects the activity of the enzyme nitrate reductase, which is a key enzyme in nitrogen metabolism that converts inorganic nitrogen (nitrate) into organic forms essential for protein synthesis thereby impacting protein production in leguminous crops during grain filling and reproductive phases (Farooq et al. 2017). Under heat stress conditions mungbean's ability to fix nitrogen is reduced due to the formation of infection threads and limited root hair production. All these factors together lower seed viability, plant vigour, and seed quality mainly due to decreased synthesis and accumulation of proteins and carbohydrates, thereby decreasing the nutritional value of the seeds (Bhardwaj et al. 2023).

Terminal heat stress poses a significant challenge for mungbean cultivation in India, especially during the spring/summer seasons (Rainey and Griffiths 2005). During the kharif season, when mungbeans are intercropped with maize, pearl millet, pigeon pea, and cotton, temperatures exceeding 40 °C can significantly reduce seed yield during early growth stages. This decline is mainly attributed to factors such as pollen sterility, inadequate fertilization, and increased flower shedding, all exacerbated by high temperatures (Mehandi et al. 2019; HanumanthaRao et al. 2016). In parts of northern India mungbean is occasionally used as a spring/summer crop. In such situations, the germination is often delayed leading to sluggish growth of plants due to

comparatively lower mean night temperature of about 20 °C (Kumar et al. 2011).

To adapt to the heat stress conditions plants have developed certain morphological modifications such as early maturation, rolling of the leaves, and change in the leaf orientation (Srivastava et al. 2012). Anatomical adaptations in plants vary from species to species, certain anatomical modifications include stomatal closure to reduce water loss thereby increasing stomatal density, enlarged xylem vessels, and reduced cell size (Bhardwaj et al. 2023). The capacity of plants to withstand heat stress could be studied through changes in physiological characteristics like photosynthetic efficiency, membrane stability, and chlorophyll fluorescence (ChlF). According to the study by Sharma et al. (2016), genotypes of mungbean and lentils that can withstand high temperatures photosynthesize more efficiently than heat-sensitive genotypes. Studies have shown that greater resistance to heat stress conditions can occur as a result of increased chlorophyll fluorescence and improved membrane stability during the grain-filling stage (Stefanov et al. 2011; Gupta et al. 2013). To withstand high temperatures, plants have also developed specific biochemical defenses. These defenses include the synthesis of secondary metabolites like isoprenoids, flavonoids, and carotenoids, which function to reduce peroxidase activity and improve heat tolerance (Rivero et al. 2004). Apart from these ROS scavenging systems, which include enzymatic (like APX, CAT, and SOD) and non-enzymatic (like glutathione and ascorbic acid) antioxidants, are the most effective and well-known defence mechanisms employed by plants to deal with oxidative stress brought on by high temperatures (Awasthi et al. 2015). The level of these antioxidants can be increased by supplementation with Ca, K, Mg, and N which can help bolster antioxidant defenses in plant cells (Waraich et al. 2012). Additionally, heat-shock proteins (HSPs) play a significant role in helping plants cope with heat stress by preventing protein denaturation via protein folding or assistance, translocation, translation, proteolysis, aggregation inhibition, and the restoration of denatured proteins (Wahid et al. 2007; Mittler et al. 2012).

So far, no significant molecular research has been carried out to elucidate the mechanisms associated with heat tolerance in mungbean, therefore an accurate representation of the function and action of genes against heat stress remains elusive. It appears that the functional significance of genes/gene products conveying tolerance needs to be clarified at the molecular level in addition to making use of genetic variation for heat stress tolerance in different legumes (HanumanthaRao et al. 2016). The current high-yielding genotypes of different legumes can be tested for heat tolerance by sowing the seeds in hot places or late in the growing season and then choosing offspring based on their development and yield characteristics. Methods that can combine high-throughput genotyping, marker-assisted selection, and phenotyping would be essential in revealing the mechanisms underlying heat tolerance. This might help open the door to the development of novel breeding techniques for developing mungbean varieties that will be heat-tolerant.

6.2 Salinity Stress

Salinity stress leads to a substantial decrease in mungbean yield due to a decline in the germination of seeds, reduced shoot and root lengths, biomass, and seedling vigour (Promila and Kumar 2000). Mungbean cultivars typically display variable levels of salinity tolerance, with certain varieties able to withstand salt concentrations ranging from 9 to 18 mmhos/cm during germination, compared to the normal concentration of 5 to 6 mmhos/cm. The germination and seedling length of all mungbean cultivars were observed to be delayed or decreased, with variations in salinity tolerance limits across different cultivars. The impact of salinity on germination and seedling development varied depending on the specific cultivar, highlighting the diverse responses of mungbean varieties to saline conditions.

The early stages of plant growth such as seed germination and survival of early seedlings are highly susceptible to the adverse effects of salinity (Sehrawat et al. 2014). Salinity stress causes impaired root development, reduced biomass accumulation, delayed flowering, increased flower shedding, and decreased yield potential thereby detrimentally altering the mungbean traits essential for successful growth and productivity as well as compromising its resilience and harvest quality. The vegetative growth stage is also highly sensitive to salinity, which leads to reduced water uptake, ion imbalance, nutrient deficiencies, oxidative stress, stunted growth, and leaf damage. These effects result in impaired growth, decreased photosynthetic efficiency, and reduced yield potential. Effective management strategies are crucial to mitigate the hazardous effects of salinity stress on mungbean crops (Munns and Tester 2008). As a result, the reproductive stage of mungbean experiences a comparatively lower impact compared to the vegetative growth phase due to saline conditions (Sehrawat et al. 2019).

Salinity stress also influences biochemical parameters, including the distribution of ions in various parts of the plant, stomatal conductance, levels of photosynthetic pigments, accumulation of osmoprotectants, enzyme activities, and the generation and scavenging of free radicals, collectively impacting mungbean's physiological and metabolic responses (Sehrawat et al. 2015). However, these aforementioned traits exhibit variability depending on factors such as plant genotype, seasonal fluctuations, and the plant's adaptability to stress conditions (Sehrawat et al. 2015, 2019).

Saline stress not only interferes with the growth and development of the mungbean plant but also affects its symbiotic association with *Rhizobium* sp., thereby hampering its nitrogen-fixing ability. *Vigna marina*, is one of the close relatives of *V. radiata* found to grow in saline conditions but it is not possible to have a natural cross between *V. marina* and *V. radiata* (Chankaew et al. 2014; Wati 2017), however, identification of saline tolerant gene from *V. marina* and then transferring it into *V. radiata* would be feasible using genetic transformation techniques.

6.3 Dehydration Stress

Mungbean is typically cultivated in rainfed conditions characterized by low humidity, and moderate rainfall between 60 and 80 cm (Sehrawat et al. 2019).

Due to its ability to thrive in soils with limited moisture or water content, mungbean is generally recognized as a drought-tolerant crop. However, a substantial decline in soil moisture levels has adverse effects on the growth, survival, and productivity of mungbeans. The primary reason for the reduction in the mungbean yields because of dehydration stress is due to loss in dry weight of the plant, lower photosynthesis and hence harvest index. The primary reasons for the reduction in yield of mungbean due to dehydration stress are lower photosynthesis, reduced plant dry weight and harvest index (Thomas et al. 2004).

Drought stress mostly impacts the developmental stages of mungbean, leading to decreased photosynthetic rates, lower harvest index, and a drop in the total dry weight of the plants, all of which influence the yield of seeds (Thomas et al. 2004; Bangar et al. 2019). When mungbeans experience water stress, their leaf water potential drops, which reduces photosynthesis because of stomatal closure and chlorophyll degradation (Raina et al. 2019). Mungbeans normally have a high rate of transpiration during flowering, the reduction in stomatal conductance during drought stress reduces transpiration thereby affecting the reproductive stage and resulting in a significant yield reduction (Van Haeften et al. 2023). One of the key physiological characteristics of mungbean is that it can serve as an accurate indicator of changes in crop transpiration rate, leaf porosity, and stomatal conductance under drought stress conditions in the canopy temperature. Canopy temperature is directly correlated to the absorption of water by roots. Lopes and Reynolds (2010) through their study on wheat stated that a cooler canopy temperature results in a deeper rooting system and increased access to groundwater. Additionally, proline content acts as a marker for a plant's ability to withstand drought stress. Proline content usually rises during drought stress conditions and falls during the recovery phase. Plants can store proline to preserve osmotic equilibrium in their cells during periods of water scarcity. Studies have revealed that under drought stress there is an increased accumulation of proline in the leaf tissues which is majorly due to a decrease in the activity of the enzyme proline dehydrogenase (Tutlani et al. 2022). Drought also leads to an increase in leaf cell thickness attributed to the excessive deposition of lignin and cutin within the cells. Furthermore, drought triggers the generation of harmful superoxide radicals during photosynthesis, contributing to cellular damage and also leading to oxidative stress in plants (Sehrawat et al. 2019).

Evaluation of drought tolerance in mungbean varieties encompasses the assessment of leaf area index, relative water content, antioxidant enzymatic activities, osmolyte accumulation, and yield components. Drought-tolerant genotypes demonstrate minimal reduction in these parameters compared to the drought-sensitive varieties.

6.4 Low-Temperature Stress

Being a tropical pulse crop suited for semi-arid and subtropical environments, its susceptibility to chilling stress can significantly hamper its productivity when temperatures drop below a certain threshold (Kabi et al. 2017). Jan et

al. (2023) suggested that among all other abiotic stresses cold stress alone can reduce 70% of mungbean productivity. Low temperatures retard the germination process of mungbean seeds, consequently prolonging the growth and developmental stages of the plant. This delay often results in variability in crop maturation and may adversely affect the reproductive capacity of the plants. A major factor in agricultural output losses is pollen sterility, which is brought primarily by cold stress delaying heading during the reproductive stage. Cold stress also results in low tillering, stunted seedlings, and poor germination (Shafiq et al. 2012). During later stages, plant growth experiences notable deceleration, potentially leading to a reduction in flower or fruit production. Furthermore, soil temperatures below 15 °C often hinder direct seeding. Reduced chlorophyll content and yield components were the results of both short and long-term cold exposure; however, longer cold exposure had more significant effects, such as the downregulation of genes involved in the synthesis of chlorophyll and the lipid synthesis in leaves (Van Haeften et al. 2023). Plants under cold stress experience a range of morphological alterations, such as reduction in leaf growth, wilting, yellowing of the leaves, and even necrosis (Suzuki et al. 2008). The damage to the plasma membrane is the major negative effect of cold stress on plants. Plasma membrane damage is caused by dehydration brought on by cold stress and is characterized by a reduction in membrane fluidity as a result of which the plasma membrane loses its functionality. This inflexibility may cause the tonoplast and plasma membrane to rupture, allowing solutes to seep out. Cold stress thereby weakens the integrity of the membrane, which causes considerable solute leakage from the cells (Bhat et al. 2022; Sanghera et al. 2011; Hatfield and Prueger 2015; Maqbool et al. 2010). Limited research has been conducted on screening and identifying chilling-tolerant genotypes, as well as determining seedling stage characteristics that could serve as markers for selecting cold-tolerant genotypes. This severe susceptibility of mungbean to chilling stress, coupled with the absence of suitable resistant genotypes, has consequently led to a decline in mungbean productivity.

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7 Effect of Biotic Factors on Mungbean

The mungbean crop in the Asian continent and Sub-Saharan African region is heavily damaged by viral, bacterial, fungal and insect pathogens. Breeding of mungbean varieties against various pathogens is ongoing. Amongst the viral diseases, the mungbean yellow mosaic disease (MYMD) is the most significant one, which is transmitted by white fly *Bemisia tabaci* leading to an 85% crop loss in India alone (Singh et al. 2000; Karthikeyan et al. 2014). The resistance to MYMD is controlled by multiple genes (Reddy 2009; Ammavasai et al. 2004; Sandhu et al. 1985). Some mungbean accessions exhibit moderate resistance against MYMD, but this level of resistance alone is not sufficient for developing highly resistant varieties. Moderately resistant cultivars and hybrids developed from them have been used in mutation breeding programs to develop resistant varieties. This approach has led to the development of several mungbean lines with high levels of resistance against MYMD. The NM

series of mungbean varieties such as NH 92 and NM 94 developed via gamma radiation mutation breeding has shown substantial potential against MYMD (Ali et al. 1997) and they have shown resistance due to a single recessive gene (Reddy 2009). Breeding programs in India and Pakistan have also focused on developing stable MYMD-resistant lines. For example, ML1628, developed by the Punjab Agricultural University in Ludhiana, India, is reported to resist multiple species or strains of the virus causing MYMD. Overall, these efforts demonstrate ongoing progress in breeding mungbean varieties with improved resistance to MYMD. However, challenges remain, particularly in regions where specific virus strains pose a significant threat. Continued research and breeding efforts are essential for developing resilient mungbean varieties capable of withstanding the diverse challenges posed by MYMD and other diseases. In addition to MYMD, Urdbean leaf crinkle disease, caused by urdbean leaf crinkle virus (ULCV), has also emerged as another potential threat to mungbean cultivation particularly in South Asia and Southeast Asian regions (Singh et al. 1988).

Cerscospora leaf spot (CLS), powdery mildew and dry root rot are the major fungal diseases while the web blight, *Fusarium* wilt and *Alternaria* leaf spot are the minor fungal diseases affecting the mungbean crop (Ryley and Tatnell 2011; Pandey et al. 2018). Amongst these dry root rot alone causes yield loss of about 10–44% in India and Pakistan (Kaushik and Chand 1987). The minor fungal diseases *Fusarium* wilt and *Alternaria* leaf spot cause a yield loss of about 20% and 10% respectively (Anderson 1985; Maheshwari and Krishna 2013). CLS is the major cause of mungbean crop loss in Pakistan and accounts for about 97% of yield loss (Bhat et al. 2014). CLS (*Cercospora cruenta*), Powdery mildew (*Erysiphe polygoni*) anthracnose (*Colletotrichum lindemuthianum*), Macrophomina blight (*M. phaseolina*) and web blight (*R. solani*) fall under the major foliar fungal disease infecting mungbean (Ryley and Tatnell 2011; Pandey et al. 2018; Iqbal and Mukhtar 2014). Combined yield loss due to these foliar fungal diseases ranges from 23% to 96%, as observed under various field trials conducted in different parts of India, with an additional yield loss of up to 61% reported from Pakistan (Iqbal et al. 1995). Yield losses due to powdery mildew were reported from various countries, including the Philippines, Australia, and different regions of India, ranging from 20% to 100%. Some specific instances include 35% loss from Gujarat, 20–40% from Chhattisgarh and Maharashtra, and 9–50% from Uttarakhand and Uttar Pradesh in Northern India. Additionally, 100% loss was reported from Maharashtra State at the seedling stage (Pande et al. 2009; Mandhare and Suryawanshi 2008). Mungbean yield losses due to anthracnose disease were estimated in the range of 24–67% (Kulkarni 2009; Deeksha and Tripathi 2002). In the South Asian region, *Alternaria* leaf spot is of common occurrence, however, economic losses caused are negligible and amount to only 10% of yield loss, as reported from the Jammu and Kashmir region of India (Maheshwari and Krishna 2013). Web blight has been a persistent problem in both Pakistan and India for several decades. *Fusarium* wilt is a minor fungal disease of mungbean caused by *Fusarium oxysporum* / *F. solani*.

However, recently the incidence of fusarium wilts has increased and has caused a crop loss of about 80% (Kelly 2018).

The breeding for fungal resistance requires the availability of resistant germplasm and markers linked with QTL regions. Amongst the fungal diseases, the major research focus for resistance has been given to powdery mildew and CLS. The molecular markers for both have been identified. The resistance against powdery mildew is governed by both quantitative and qualitative modes (Kasettranan et al. 2010; Nair et al. 2019). In the South Asia and South East Asian regions foliar and root rot are the major fungal diseases of concern.

The wilt and root rot complex caused by dry root rot/Charcoal rot (*Macrophomina phaseolina*) and *Rhizoctonia* root rot (*Rhizoctonia solani*) are significant soil-borne vascular diseases. These diseases affect mungbean plants throughout all growth stages, causing substantial yield losses (Iqbal and Mukhtar 2014). Dry root rot and wilt can lead to substantial yield losses in mungbean crops. Reported yield losses due to dry root rot range from 11% in Northern India to as high as 44% in Pakistan (Kaushik and Chand 1987). China reported its first dry root rot disease in 2010 in Shanxi province (Zhang et al. 2011). Dry root rot and wilt can affect mungbean plants at all growth stages, from seedling to maturity. This wide-ranging impact underscores the severity of the disease and its potential to cause significant economic losses for growers.

The widespread and detrimental effects of fungal diseases on mungbean cultivation, emphasize the need for effective disease management strategies to mitigate yield losses and ensure sustainable production. Climate change is expected to intensify extreme weather events such as heavy rainfall, droughts, and storms. These events can create favourable conditions for the proliferation and spread of certain crop diseases, particularly those that thrive in moist or humid environments. Conversely, periods of drought or water stress may weaken plant's natural defenses and increase their susceptibility to infections. Climate change is likely to exacerbate the risk of crop diseases in many regions, the specific impacts can be highly variable and context-dependent. Hence, it is imperative to understand these complex interactions to develop successful ways to avert the effect of climate change on agricultural productivity and food security.

The primary bacterial diseases affecting mungbean yield are tan spot, bacterial leaf spot and halo blight. In the Indian context bacterial leaf spot has shown about 30% incidence while in China and Australia halo blight is the primary emerging bacterial disease in mungbean (Sun et al. 2017). The least information about resistance against bacterial pathogens is available in mungbean cultivars. Mungbean cultivars Jalgaon 781, P 475, P 646 and PLM 501 have been found to be resistant to bacterial leaf spot (Patel and Jindal 1972; Thakur et al. 1977). No QTL for bacterial disease has yet been identified (Nair et al. 2019).

Secondary metabolites play a significant role in resistance to pathogens. Phytic acid (PA) present in mungbean has been demonstrated to impart resistance against various pathogens. Dhole and Reddy (2016) 94 genotypes

of mungbean and found 37, 9 and 1 were found resistant to yellow mosaic disease, powdery mildew and bruchid respectively and the resistance was driven by the presence of PA amount. It was observed that seed phytic acid content varied among the mungbean genotypes. Interestingly, the genotype resistant to bruchid infestation had the highest seed phytic acid content, followed by genotypes resistant to powdery mildew and yellow mosaic disease. High seed phytic acid content (>8 mg/g) was associated with resistance to yellow mosaic disease and powdery mildew. In order to impart resistance to bruchid infestation very high seed phytic acid content (~18 mg/g) is required. Lowering the concentration of phytic acid below the threshold levels mentioned may decrease the tolerance to biotic stresses, even in the presence of resistance genes in the plant (Dhole and Reddy 2016). Understanding the relationship between seed composition and stress tolerance could contribute to the development of breeding strategies aimed at improving stress resilience in mungbean cultivars.

Germplasm has been crucial in the advancement of numerous mungbean varieties. The process of gathering, assessing, and defining trait-specific germplasm requires a structured allocation of both time and financial resources. This makes sure that the complete potential of germplasm can be productively used to address specific trait requirements and it can enhance the cultivar development. The mungbean minicore collection has been provided to the partner countries of the International Mungbean Improvement Network (IMIN), which is funded by the Australian Center for International Agricultural Research (ACIAR) (Schafleitner et al. 2016). This germplasm needs to undergo exact screening for various biotic stresses and can be utilized in introgression breeding to create cultivars that are resistant to these various stresses. The mungbean minicore collection is valuable for identifying new traits for future breeding programs. The collection comprises a significant genetic resource for mungbean breeding programs. Further, the necessary materials for breeding have also been prepared at the Indian Council for Agricultural Research (ICAR)—Indian Institute of Pulses Research (IIPR) in Kanpur, the Department of Agricultural Research (DAR) in Myanmar, Bangladesh Agricultural Research Institute (BARI) in Bangladesh, and World Vegetable Center in Hyderabad. These materials were therefore developed using mungbean minicore accessions. Although creating biotic stress-resistant varieties is crucial, similarly educating mungbean farmers on best practices is equally important. To make mungbean a significant pulse crop and address global protein and calorie malnutrition, it is essential to teach poor and marginal farmers how to cultivate resistant varieties and manage crops effectively. The summary of the published literature about the studies conducted on *V. radiata* related to abiotic and biotic stress conditions. It also shows the variety/cultivar used in the study and possible aftereffects under stress conditions (Table 1).

Table 1 Effect of various abiotic and biotic stress factors on *Vigna radiata* at various developmental stages

Stress factor/causative agent	Variety/cultivar	Stress specification	Effect on the stage of plant growth	Characteristic	Reference
Abiotic stress					

Stress factor/causative agent	Variety/cultivar	Stress specification	Effect on the stage of plant growth	Characteristic	Reference
High-temperature stress/heat stress	SML 832 and 668	>40/25 °C day/night	Reproductive stage, flowering, and podding stage.	Reduction in seed yield, biomass, filled pods, pod set, and seed number. Reduction of pollen germination, pollen viability, pollen load, pollen tube growth and stigma receptivity. And inhibition of chlorophyll concentration, photosynthetic efficiency and stomatal conductance in leaves as well as a drop in sucrose concentration in the leaves and anther.	Kaur et al. (2015)

Stress factor/causative agent	Variety/cultivar	Stress specification	Effect on the stage of plant growth	Characteristic	Reference
	Heat-tolerant [HT: EC 693369] and Heat-sensitive [HS: KPS1]	42 °C/30 °C	Seed-filling stage	Reduction in seed yield, weight, and nutritional quality due to reduced time duration and seed-filling rate. Decrease in starch, protein including storage protein, fat, and mineral contents. Severe damage to the leaves, such as a decrease in the water content of the leaves, diminished fluorescence, and damage to the membranes.	Priya et al. (2023)
	IPM 02-3, IPM 02-14, MH 2-15, MH 125, MH 318, SML 832, MH 421, MH 1142, PM-5, PDM 139, IPM 410-3, PDM 54 and IPM 205-7	38–40 °C	–	Significant effect on the seed composition, protein content, and quality. Less damage in tolerant plants in comparison to the sensitive genotype.	Batra et al. (2023)
	Harsha, EC693369, EC693357, ML1299 and EC693358	<40/28 °C	Reproductive stage	Leaves with symptoms of leaf rolling, scorching, and chlorosis. Reduction in pods, biomass, flowers, leaf area, and seed yield was observed due to shortening of flowering time and podding duration.	Sharma et al. (2016)

Stress factor/causative agent	Variety/cultivar	Stress specification	Effect on the stage of plant growth	Characteristic	Reference
Low-temperature stress/cold stress	NM94 and VC1973A	4 °C—3 days	–	Reduced relative growth rate, decreased PSII photochemical efficiency, and DPPH scavenging activity, altered ultrastructure, and elevated expression of chilling-regulated genes (CORs)	Chen et al. (2017)
	204 Genotypes, 4 high yielding released varieties (C1-C4), 58 Odisha landraces, 15 recombinant Inbred lines (RIL), 127 Indian varieties (G1-G200)	10 °C—8 days	Seedling, vegetative, and reproductive stage	Decrease in germination percentage, germination index, and seedling length. Significant reduction in the plant height, number of pods/plant, yield/plant, and seed weight.	Manasa et al. (2023)
	Lvfeng 2 and Lvfeng 5	15 °C—(1–4 days)	Flowering stage	Decreased yield, low chlorophyll content and downregulation of chlorophyll biosynthesis and leaf cuticle lipid synthesis genes.	Hu et al. (2022)

Stress factor/causative agent	Variety/cultivar	Stress specification	Effect on the stage of plant growth	Characteristic	Reference
Dehydration stress/drought stress	TMB 37, SGC 16, Pratap and SG 21-5	Irrigation withdrawal for 15 consecutive days	Vegetative growth stage, flowering stage, pod-filling stage	Reduction in leaf water potential as well as decrease in photosynthesis as a result of stomatal closure and chlorophyll degradation.	Baroowa et al. (2016)

Stress factor/causative agent	Variety/cultivar	Stress specification	Effect on the stage of plant growth	Characteristic	Reference
	Partow, Barymung-2, vc6368	Different times of water withholding	Vegetative and reproductive growth stage	Reduction in plant height, biomass, kernel weight, and a significant decrease in harvest index. Increase in seed protein content during the vegetative and flowering stage.	Sadeghipour (2009)
	Kawmay-1, VC-2010 and King	Four irrigation intervals (3-, 5-, 7-, and 9-day intervals)	3 weeks after sowing	The amount of irrigation had an impact on biological yield, seed yield, shoot weight, plant height, harvest index, and water usage efficiency. The yield and yield components were hindered by the water stress.	Ahmad et al. (2015)
	Tvr79, Tvr19, Tvr21, Tvr97, Tvr32, Tvr40, Tvr29, Tvr42, Tvr43, Tvr46, Tvr44, Tvr47, Tvr48, Tvr62, Tvr49, Tvr82, Tvr14, Tvr17	20% Polyethylene glycol (PEG)-6000	Early vegetative growth stage	All varieties showed more that 60% germination. The treated and control shows similar leaf rolling morphology. The H_2O_2, O^{2-}, and MDA content was found to be higher under drought stress conditions.	Abiala et al. (2022)
	NM-13-1^{Tol} (Drought Tolerant) and NM-54^{Sens} (Drought Sensitive)	Withholding water for 2 weeks	2-week-old young plants	A greater reduction in growth and decrease in PSII efficiency in the drought-sensitive cultivar.	Bano et al. (2021)

Stress factor/causative agent	Variety/cultivar	Stress specification	Effect on the stage of plant growth	Characteristic	Reference
Saline stress	Pusa vishal and Pusa ratna	Control- 0 mM NaCl, 50 mM NaCl (Treatment1), and 75 mM NaCl (Treatment2)	Vegetative and reproductive growth stage- summer and spring season	Decrease in the plant's height, length, leaf area, rate of photosynthesis, total chlorophyll and carotenoid levels, number of pods per plant, and grain production. Plants in the early vegetative stage were found more resistant to salt stress as compared to plants in the late vegetative and reproductive stage.	Sehrawat et al. (2015)

Stress factor/causative agent	Variety/cultivar	Stress specification	Effect on the stage of plant growth	Characteristic	Reference
	cv. J-45	Control- distilled water, 60 mM/L, 120 mM/L, 180 mM/L NaCl	–	Reduced and delayed/inhibited radicle and hypocotyl elongation respectively. Reduction in fresh and dry masses and water content of the embryo axes. Increased net leakage of potassium (K) into the media over time with higher NaCl concentrations.	Promila and Kumar (2000)
	BARI Mung-6, Binamoog-5, Tila Mung, and Binamoog-8	Control- distilled water, (40, 80, 120, 160 mM/L NaCl)	Germination and early growth stage	Delay in germination and increase in Mean Germination Time with increasing salt concentration. Reduction in the total plant growth due to decrease in shoot and root length.	Podder et al. (2020)
	–	0 mM, 50 mM, 100 mM, 150 mM NaCl	Salt stress treatment was provided on the 13th day of growth for 24 h. The plants were rehydrated and the NaCl solution was removed after 24 h.	Reduction in the amount of K+ ions, chlorophyll and carotenoid pigments, fresh and dry weight, and relative water content. Na+ ion and Na+/K+ ratios in the roots, stem, and leaves significantly increased. Stressed leaves have higher levels of lipid peroxidation and peroxide. Stressed roots, stems, and leaves have higher concentrations of	Panda and Khan (2009)

Stress factor/causative agent	Variety/cultivar	Stress specification	Effect on the stage of plant growth	Characteristic	Reference
				proline and ascorbate. A decrease in protein content and activity of the enzymes CAT, SOD, POX, APX, and GR in the root.	
Biotic stress					
Pseudomonas syringae pv. *Phaseolicola,* Halo blight	*Vigna radiata* L. Wilczek var. radiate, Australian	Bacterial	Leaf tissues	Substantial yield loss and unpredictability in production Lesions merge together with surrounding necrotic leaf patches or age naturally	Noble et al. (2019)
Frankliniella schultzie	Wild mungbean	Viral	Leaf	It displays venial necrosis, chlorosis on the lamina, downward curling of the leaves, and apical necrosis, which kills the plant.	Masoodi et al. (2018)
Bruchid (*Callosobruchus chinensis L.*)	*Vigna radiata* var. sublobata	Insect	Seed	Decreased levels of phytotic acid (PA) in seeds, which increases their vulnerability to disease	Dhole and Reddy (2016)
Stem fly (*Ophiomyia* spp.)	BM 4, PKV AKM-4, BM 2011-1, AKM 12-14, AKM 12-23, PHULE M 702-1, PKV GREEN GOLD, VAIBHAV, AKM 12-24 and BPMR 145	Insect	Stem, pod, leaf	Plant growth is hampered by the loss of cell sap.	Bhople et al. (2017)
Anthracnose *Cercospora* leaf spot (CLS) (*Cercospora* sp.)	KMP-13	Fungal	Leaf	It impacts the defoliation of leaves caused by big coalescing lesions.	Bhaskar (2017)

Stress factor/causative agent	Variety/cultivar	Stress specification	Effect on the stage of plant growth	Characteristic	Reference
Powdery mildew (*Maruca. vitrata, Clavigralla. gibbosa, Riptortus. pedestris*)	C1-28-20, C1-32-22, C1-34-23, C1-37-23, C1-44-31, C1-175-111, C1-38-27, C1-275-177, C1-246-159, C1-236-152, C1-41-28	Fungal	Pod	Spotted pod borer damage to pods on several genotypes.	Kumar and Singh (2017)

“–” meaning no information is available

8 Economic Significance

Mungbean is primarily cultivated in Asia, with approximately six million hectares devoted to its production annually. Despite its widespread cultivation, the average yield remains relatively low at around 400 kg per hectare. It is recognized for its high dietary quality, providing significant amounts of carbohydrates, protein and various micronutrients essential for human health. It contains the essential amino acid lysine, which is often deficient in cereal-based diets. However, mungbean is relatively low in methionine, another essential amino acid found in cereals. Mungbeans are rich in antioxidants, which play a crucial role in scavenging free radicals and protecting cells from oxidative damage. The high antioxidant activity of mungbean contributes to their potential health benefits. Increased consumption of mungbean has been associated with improved health outcomes, particularly in women, in several Asian countries (Shi et al. 2016). Between 1984 and 2006, as mungbean consumption rose, there was a corresponding improvement in the health of women. This positive correlation suggests that incorporating mungbean into diets may lead to better health outcomes and potentially significant economic benefits. In a nutshell, the nutritional composition and health benefits of mungbean make it a valuable crop with the potential to address dietary deficiencies and improve human health, particularly in regions where cereal-based diets predominate. Increasing productivity and promoting the consumption of mungbean could have positive implications for both nutrition and economic well-being, especially in developing countries where mungbean is a staple food (Shanmugasundaram et al. 2010). The sprouts are a rich source of vitamins and minerals, which makes them a sought-after choice for health-conscious consumers and the cultivation of mungbean sprouts requires minimal space and resources, making it accessible to a wide range of growers, including small-scale producers (Singh and Singh 1995; Kyriacou et al. 2016). Mungbean sprouts are marketed in different forms, including fresh, canned, and processed, catering to diverse consumer preferences. Europe has seen a significant increase in mungbean imports over the past 5 years, primarily for

sprout production. The main import origins are Myanmar, China, and Australia, with the United Kingdom being the largest importer, likely due to its large Indian and Pakistani populations (Eurostat 2018). Mungbean cultivation not only provides income through the sale of beans but also offers benefits such as reducing farm inputs for subsequent crops. For example, when intercropped between wheat and rice in India, mungbean leaves nitrogen in the soil for the following crop, contributing to soil fertility and reducing the need for nitrogen fertilizers. Crop rotation involving mungbean cultivation has been observed to enhance the yields of subsequent crops (Sekhon et al. 2006). For instance, studies have shown significant increases in sorghum yields after mungbean cultivation, attributed to nitrogen fixation by mungbean and its positive impact on soil fertility.

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9 *Vigna radiata*: Minor to Major Cereal

The world population has increased rapidly over the last few centuries and is expected to increase by nearly 2 billion in the next 30 years (Bongaarts 2009). With the expanding population, there is a rising demand for food. Over the past few years to meet the nutrient requirements pressure has been exerted on food crops such as rice, wheat, maize, and potato (Kamanga and Mndala 2019). To reduce the pressure on these major food crops there is a necessity for the introduction of marginal/minor crops like mungbean into the agricultural system. In doing so, along with meeting the adequate nutrient requirement mungbean cultivation can also contribute to food security and economic livelihoods in many regions, particularly in developing countries where it serves as a staple food crop and also a major income source for smallholder farmers (Baza et al. 2022).

Beyond their agronomic benefits, mungbean offer substantial nutritional advantages. The popularity of mungbean consumption alongside cereals is increasing due to several appealing characteristics, including easily digestible proteins, a pleasant taste, and low-flatulence soluble carbohydrates (Kudre et al. 2013). Mungbean seeds and sprouts are low in calories compared to other cereals and offer various health benefits, especially when germinated. These benefits include maintaining gut microbial flora, reducing the absorption of toxins, and preventing metabolic disorders (Huang et al. 2014). Mungbean contains phytic acid, tannins, and other compounds with biological activities. Recent studies have identified bioactive compounds in mungbean with health-promoting effects (Ganesan and Xu 2018), such as organic acids, flavonoids, and phenolic compounds. These compounds have detoxifying, diuretic, anti-inflammatory, and anticancer properties, among others (Shi et al. 2016; Kim et al. 2012). Mungbean has been traditionally used for various ailments like acne, fever, liver diseases, and nervous disorders (Tang et al. 2014). They are a rich source of protein, essential amino acids, vitamins (such as vitamin B6 and folate), dietary fiber, and minerals (including iron, potassium, and magnesium). Incorporating mungbeans into diets can help combat malnutrition and diet-related health issues prevalent in many regions. Apart from human consumption, mungbean is also used as a fodder crop. The

haulms are directly used as fodder and the husks of the beans are used as feed concentrate. It is also grown as a cover crop, green manure and hay. Due to the less hairy leaves and stems, mungbean serves as a better hay than urad bean.

Mungbeans, with their adaptability to various ecological conditions and minimal resource requirements, can also help meet nutritional demands thereby contributing to food security, particularly in developing nations where they serve as both a dietary staple and a significant income source for smallholder farmers (Baza et al. 2022). The cultivation of mungbeans has the potential to enhance agricultural resilience. Mungbeans thrive in diverse environments, including marginal lands with low fertility or limited access to irrigation. This resilience can be a game-changer for farmers facing challenges like climate variability or water scarcity. Furthermore, mungbeans enrich soil health by fixing atmospheric nitrogen, reducing the need for chemical fertilizers, and promoting sustainable agricultural practices.

In socio-economic terms, the cultivation of mungbeans presents opportunities for income diversification and poverty alleviation. Their relatively short growth cycle and low input requirements make them attractive for smallholder farmers who may lack resources for intensive agricultural practices. The sale of mungbeans in local and international markets can generate additional income streams, contributing to economic stability and rural development.

Mungbean cultivation should be promoted through targeted support, including access to improved seed varieties, agronomic training, market linkages, and financial incentives. By integrating mungbeans into crop rotations and diversifying agricultural landscapes, stakeholders can enhance food system resilience, reduce pressure on major food crops, and foster sustainable food production pathways for a growing global population.

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10 Empowering Women in Rural Areas

Women make substantial contributions to the agricultural labour force, accounting for 36.7% in developed countries and 43.6% in developing countries (Luqman et al. 2006). Their involvement spans various aspects of agricultural activities, including crop production, livestock management, and cottage industries. Women often dedicate a significant portion of their time to agricultural tasks, spending two-thirds of their time on activities such as crop production, poultry farming, and livestock management. They also play a significant role in food storage and processing, highlighting their importance in ensuring food security and preservation (ESCAP 1997; Jamali 2009). Mungbean cultivation involves labour-intensive tasks, with farmers spending a considerable amount of time on activities such as harvesting, threshing, and seed cleaning. These tasks, which account for a significant portion of total working time, are predominantly carried out by women and children within farming families (Sisei 2016). Women and children actively participate in almost every stage of mungbean production, except ploughing. Their involvement underscores the crucial role they play in agriculture, with

mungbean often recognized as “women’s crops” in many smallholder farming communities (Doss 2002). Although the income generated from mungbean cultivation may be marginal, it plays an important role in supplementing household needs and contributing to the overall well-being of farming families. The cash flow from mungbean cultivation can help improve access to health care and education, thereby enhancing the socio-economic status of rural households (Kalansooriya and Chandrakumara 2016).

Women in mungbean production areas are often engaged in tasks that require less physical strength, such as clearing fields, grading seeds, weeding, harvesting, and post-harvest management. Despite their significant contributions, these activities are considered less prestigious and are added to their existing responsibilities of housekeeping, childcare, and livestock management (Satyavathi et al. 2010). It has been observed that generally, women work heftier and longer duration than men, encompassing diverse pursuits. However, more workload does not necessarily increase their ability to produce more food, earn enhanced income, or care for the family. This situation may result in changes in cooking attributes, leading to the preparation of fewer and less nutritious meals (FAO Rome). Traditional gender norms and systems often limit women’s access to resources and opportunities, perpetuating a gendered division of labour where women are allocated the most labour-intensive and poorly rewarded tasks. This imbalance can hinder women’s economic empowerment and overall well-being. Mechanization in mungbean production could alleviate some of the burdens faced by women by freeing up their time for other productive activities, such as self-employment, value addition, income generation, and family care. By reducing manual labour requirements, mechanization could also lead to increased mungbean production, improved incomes, and potentially more land could be brought under cultivation to meet market demand (Diao et al. 2014).

Women in the rural setup are integral to agricultural production and food systems, contributing significantly to food security, livelihoods, and rural economies. Recognizing and supporting their role is crucial for sustainable agricultural development and empowering rural communities. Recognizing the contributions of women and children to mungbean cultivation is essential for fostering inclusive and sustainable agricultural development in the country.

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11 Challenges in Mungbean Production

In spite of having multiple nutritional and agricultural benefits, such as a pillar summer or spring crop, less cultivation cost, short duration, photoperiod insensitivity, good water use efficiency, suitable for crop rotation, and maintaining soil fertility, the production of mungbean faces many challenges (Bibiso 2016). These primarily include poor-quality seeds, a lack of better varieties, environmental conditions, pathogens, poor husbandry practices and losses during post-harvest processing. Bruchids, specifically *Callosobruchus* sp., pose a significant threat to stored mungbean grains, causing extensive damage if left unchecked. Bruchids lay their eggs on mungbean pods in the field. Upon hatching, the larvae develop within a single bean until reaching

adulthood. Adult beetles then lay eggs on other beans, perpetuating the cycle. During storage, bruchids can rapidly infest and destroy stored mungbean grains, rendering them unfit for consumption. Some mungbean genotypes possess inherent genetic resistance against bruchids. This resistance has been identified and utilized in breeding programs to develop mungbean varieties with enhanced resistance to bruchid infestation. Breeding programs in China, Korea, and at institutions like the WorldVeg have focused on incorporating genetic resistance to bruchids into mungbean varieties. By selecting and crossing mungbean lines with natural resistance, breeders have developed varieties that exhibit increased tolerance or resistance to bruchid infestation. Genetic studies have mapped the genes responsible for bruchid resistance in different mungbean sources. This knowledge has facilitated the development of molecular markers that enable breeders to select for bruchid-resistant traits more efficiently in breeding programs. Marker-assisted selection (MAS) allows breeders to identify and incorporate desirable traits into new mungbean varieties with greater precision and speed. Breeding for bruchid-resistant mungbean varieties offers an effective and sustainable solution to mitigate post-harvest losses caused by this storage pest. By harnessing natural genetic resistance and leveraging modern breeding techniques, researchers and breeders aim to develop mungbean varieties that are resilient to bruchid infestation, ensuring the quality and safety of stored grains for consumption and marketability. These problems are further supplemented by weed and pest management, the perception of the grower, and the lack of a vast acceptable market (AMA 2015; Bibiso 2016).

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12 Mungbean in Sustainable Agriculture

In the current scenario, sustainable agriculture is the key to balanced development. Sustainable agriculture seeks to create resilient, environmentally sound, socially equitable, and economically viable food systems that can meet the needs of present and future generations.

Mungbean can fix atmospheric nitrogen via symbiotic association. This makes it a valuable component of sustainable agriculture, especially in settings where conservation agriculture and water-saving techniques are employed. Integrating mungbean into conservation agriculture systems, along with best management practices, can improve the sustainability and productivity of cereal-based cropping systems, such as maize-wheat rotations in the Eastern Indo-Gangetic Plains of India (Laik et al. 2014; Parihar et al. 2017). Introducing mungbean into crop rotations can further enhance the total biomass productivity, water and energy use efficiency. Long-term experiments conducted in India demonstrate that incorporating mungbean into rotations such as maize-wheat-mungbean and maize-chickpea-sesbania can lead to increased nutrient uptake, productivity and profitability, particularly in the north-western parts of India and in similar agro-climatic situations (Yadav et al. 2016; Pataczek et al. 2018).

The integration of mungbean into cereal cropping systems, especially in conjunction with conservation agriculture practices, offers several benefits for

soil health, nutrient availability, and overall agricultural sustainability. Among the conservation agriculture-based systems evaluated, the maize-wheat-mungbean system emerged as the best alternative option for achieving sustainable productivity and this has led to conserving the natural resources and improving the soil quality index by a factor of 35%. A similar outcome was observed in Bangladesh at the High Ganges River Floodplain. Minimal disturbance of soil, the inclusion of legume/green manure crops into rice-wheat cropping systems, and retention of crop residues *in situ* have further contributed to enhanced soil carbon (C) status, improving the properties of soil and leading to maximizing the grain yields (Dey et al. 2016). Integrating mungbean into cereal cropping systems, along with conservation agriculture practices, significantly increased the levels of extractable zinc and manganese in the soil compared to traditional farming without conservation agriculture. This suggests that mungbean integration can enhance soil nutrient availability, potentially reducing the need for external fertilizer inputs. The study also demonstrated a significant saving of nitrogen (30%) and potassium (50%) in wheat crops after 4 years of mungbean integration in conservation agriculture systems. This indicates that conservation agriculture, combined with mungbean integration, can improve soil properties and nutrient availability, leading to reduced reliance on external fertilizer inputs over time (Jat et al. 2018). Recent studies performed at the Indo-Gangetic Plains have shown that integrating mungbean into maize-wheat or rice-wheat or cropping systems, alongside conservation agriculture measures and precision irrigation, can improve water and radiation use efficiency. This highlights the potential of mungbean integration to enhance resource utilization and crop productivity in water-limited environments (Parihar et al. 2017). Among the tested rotations, a mungbean-chickpea sequence yielded the highest net benefit values and cost-benefit ratios, indicating its economic viability and potential for enhancing agricultural sustainability in dryland regions (Hassan et al. 2016).

Overall, the integration of mungbean into cereal cropping systems, particularly when combined with conservation agriculture practices, holds promise for improving soil health, nutrient availability, water and radiation use efficiency, and overall agricultural sustainability in diverse agroecological contexts. Further research and adoption of these practices could contribute to more resilient and productive agricultural systems, particularly in regions facing challenges such as water scarcity and nutrient depletion.

13 Conclusion

The advantages, and problems related to mungbean farming, and ways to mitigate those problems has been summarized (Fig. 1). Despite addressing knowledge gaps in breeding against pests and diseases, the lack of improvement in the abiotic stress tolerance of mungbean remains a concern. Future research should focus on developing novel breeding approaches to enhance mungbean's ability to tolerate saline conditions. There is a need to explore the potential of mungbean in addressing hidden hunger and linking it

with national food policies. This would not only contribute to improving farm incomes but also necessitate shifts in on-farm strategies in order to bridge the gender disparity, especially considering the current manual harvesting practice. Mungbean often exhibits a low nitrogen harvest index, as most biologically fixed nitrogen is removed from the field by the grains. The use of plant growth-promoting rhizobacteria (PGPR) could substantially improve the efficiency of biological nitrogen fixation, particularly with access to local rhizobacterial information. Integrating PGPR with conservation agriculture measures could help mitigate soil deterioration while improving and sustaining crop productivity.

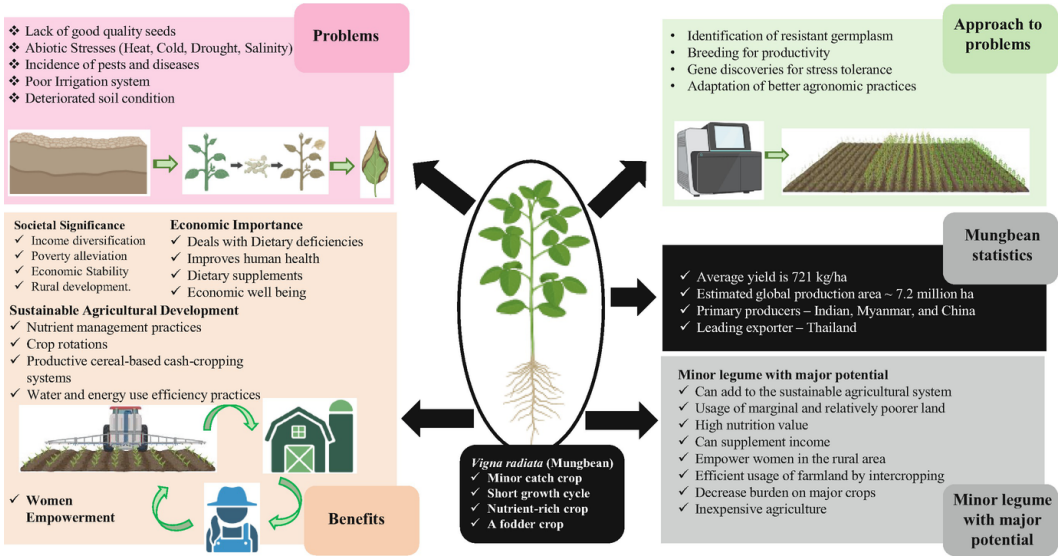


Fig. 1 Overall architecture of the chapter in a nutshell. The various problems faced by mungbean farming and the probable ways to address them, its benefits and how it can be brought to the mainstream agricultural system. Pictures used are for representation purposes only

Despite available knowledge, there's little emphasis on adopting conservation agriculture practices in South Asia, particularly in the Indian subcontinent. Promoting the use of conservation agriculture could be pivotal in addressing soil degradation and enhancing mungbean productivity. There is a knowledge gap regarding the effects of mungbean-dependent relay cropping in cereal-based cash crop systems. Future research should focus on understanding the potential benefits and challenges of integrating mungbean into existing cropping systems. Crop management options that encourage integrating legumes like mungbean into the existing cropping systems could help mitigate the impact of erratic monsoon rains in rainfed-dependent ecosystems. This highlights the importance of diversifying cropping systems to enhance resilience to climate variability. Addressing these research and development priorities could contribute to enhancing the resilience, productivity, and sustainability of mungbean cultivation, particularly in regions vulnerable to environmental stresses and food insecurity.

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Approaches to Enhance Abiotic and Biotic Stress Tolerance in Leguminous Crops and Microgreens

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Keywords Abiotic and biotic stressors – Legume crops resilience – Microgreens – Omics approaches – New Breeding techniques

Abbreviations

AM Association Mapping

ATFCC Australian Temperate Field Crops Collection

CEMS Capillary Electrophoresis–Mass Spectrometry

CG Candidate Gene

CGIAR Consultative Group on International Agricultural Research on Resources

CIAT International Center for Tropical Agriculture

circRNA circular RNA

CRISPR-Cas Clustered Regularly Interspaced Short Palindromic Repeats-CRISPR-associated protein

DEGs Differentially Expressed Genes

DEPs Differentially Expressed Proteins

DGE Digital Gene Expression

DGTP Direct Gene Transfer to Protoplast

DMRs Differentially Methylated Regions

EP Electroporation

ESTs Expressed Sequence Tags

FAO Food and Agriculture Organization

GC–MS Gas Chromatography–Mass Spectrometry

GM Genetically Modified

GS Genomic Selection

GT Genetic Transformation

GWAS Genome-Wide Association Studies

HTP High Throughput Phenotyping

IBPGR International Board for Plant Genetic Resources

ICARDA International Center for Agricultural Research in the Dry Areas
ICRISAT International Crops Research Institute for the Semi-Arid Tropics
IITA International Institute of Tropical Agriculture
Indels Insertions/Deletions
KASP™ Kompetitive Allele-Specific Polymerase Chain Reaction
lncRNAs long noncoding RNAs
MABC Marker-assisted Backcrossing
MAGIC Multiparent Advanced Generation Inter-Crossing
MAS Marker Assisted Selection
miRNAs microRNAs
MP Microprojectile Bombardment
MS Mass Spectrometry
MYMIV Mungbean Yellow Mosaic India Virus
NBTs New Breeding Techniques
ncRNAs Noncoding RNAs
NGS Next Generation Sequencing
NIRS Near-Infrared Spectroscopy
ODM Oligonucleotide Directed Mutagenesis
PPI Protein-Protein Interaction
QC Quality Control
QTL Quantitative Trait Loci
RAPD Randomly Amplified Polymorphic DNA
RdDM RNA-dependent DNA Methylation
RFLP Restriction Fragment Length Polymorphism
RNAi RNA interference
ROS Reactive Oxygen Species
SAGE Serial Analysis of Gene Expression
siRNAs small interfering RNAs
snoRNAs small nucleolar RNAs
SNPs Single Nucleotide Polymorphisms
SRM Selected Reaction Monitoring
SSN Sequence Specific Nuclease
SSRs Simple Sequence Repeats
TALENs Transcription Activator-like Effector Nucleases
TFs Transcription Factors
UV UltraViolet
ZFNs Zinc Finger Nucleases

1 Introduction

Legumes are members of the third largest flowering plant family, Fabaceae, alternatively known as Leguminosae. Being the nutritional banks cultivated primarily for human consumption, livestock feed, and soil-enhancing green manure, they rank second in global consumption, only preceded by the grass family, Gramineae (Poaceae) (Çakir et al. 2018). Some economically significant and widely cultivated legume species include soybeans *(Glycine max)*, peanuts (*Arachis hypogaea*), lentils (*Lens culinaris*), chickpeas (*Cicer arietinum*), beans (*Phaseolus vulgaris*), clover (*Trifolium* spp.), tamarind (*Tamarindus indica*), alfalfa (*Medicago truncatula*), lupins (*Lupinus perennis*), carob (*Ceratonia siliqua*), and mesquite (*Prosopis* spp.) (Biswas et al. 2023). These are globally acclaimed staple foods with a wealth of nutritional and medicinal benefits, providing vital protein, vitamins, carbohydrates, and fiber supporting a healthy lifestyle (Mefleh et al. 2021). As plant foods, legumes have a low saturated fat content and are also cholesterol free, making them essential components of the diet regimens of patients with chronic maladies, diabetes, obesity, and cardiovascular diseases (Mudryj et al. 2014; Marventano et al. 2016; Clemente and Olias 2017; Kim et al. 2016). Individuals prioritizing health increasingly favor the inclusion of plant-based proteins in their diets, including Mediterranean diets and vegetarian and vegan diets, over animal-derived protein sources, a phenomenon observable even in developing nations (Ferrari et al. 2022; Polak et al. 2015).

Leguminous microgreens, which are 7–21-day old seedlings derived from these nutrient rich legumes, including beans, lentils, peas, alfalfa, and more, are considered “functional foods/super food” with nutritional and antioxidant properties, containing health promoting and disease preventing properties. Microgreens are approximately five times nutritionally richer than their mature stages; additionally, they generate less food waste (Gupta et al. 2023; Tallei et al. 2023; Priti et al. 2021). The nutritional value comes from bioactive compounds, phytochemicals, and antioxidants, including proteins, vitamins, minerals, phenols, carotenoids, and glucosinolates Microgreens are gaining popularity because of multiple factors that include nutritional content, high yield, rapid production, and appealing aroma (Gupta et al. 2023). In addition, leguminous crops play multifaceted roles in agriculture, which aligns well with sustainable agricultural practices. They are used as green manure, animal feed, and forage, for the extraction of vegetable oil, gums, and dyes, and to prevent soil erosion. When incorporated into crop rotation systems with cereals and other crops, they significantly reduce greenhouse gas emissions and nitrogen run-offs by fixing atmospheric nitrogen and also improve soil’s nutritional quality and fertility (Kebede 2021; Sánchez-Navarro et al. 2020; Stagnari et al. 2017). Therefore, by enhancing nutrition, supporting sustainable agricultural practices, and offering numerous health benefits, leguminous microgreens and crops have the potential to improve food systems.

Legumes are found in various life forms distributed across the globe, ranging from herbs in arctic alpine regions (e.g., *Lupinus arcticus; Trifolium alpestre*), perennial shrubs in temperate and tropical regions (e.g., *Medicago spp., Desmodium intortum*); to annual xerophytes (e.g., *Prosopis spp.*), and equatorial giant trees (e.g., *Hydrochorea marginata*) (Smýkal et al. 2014). Legume crops

growing in such diverse growth conditions are vulnerable to challenges posed by environmental and biological stressors, which significantly impact plant survival, crop productivity, and thereby overall production (Leitão et al. 2021). Abiotic stressors include drought, erratic rainfall, salinity, extreme temperature conditions, heavy metal and pesticide contamination, harmful UV radiation, and nutrient deficiency (Araújo et al. 2014). Major diseases impacting legumes include fungal diseases such as powdery and downy mildews, charcoal, collar and wet root rots, alternaria, ascochyta and phytophthora blights, Botrytis gray molds, fusarium and vascular wilts, and rusts, bacterial diseases including blights and mosaics; and damages caused by cyst and root-knot nematodes, parasitic weeds, and chewing/sap-sucking insects like pod borers/whitefly (Darai 2017; Newton et al. 2011). These stressors, whether acting in isolation or in combination, affect a plant's cellular homeostasis, which is the main cause of compromised plant growth and survival, affecting crop productivity (Sandhu et al. 2023; Latef and Ahmad 2015; Dita et al. 2006). Consequently, these stressors can have cascading effects on the yield, quality, and nutritional content of the microgreens derived from these leguminous crops. Additionally, cultivating plants with enhanced nutrient profiles can yield microgreens with substantial biomass within a short growth period. In response to these abiotic and biotic challenges, plants must modulate a variety of processes, including intricate gene interactions and cross-talk with many pathways at the molecular, cellular, physiological, and biochemical levels. It is essential to comprehend these complex dynamics in order to design strategies that will increase stress resilience and, thereby, crop productivity. A deep understanding of these mechanisms is crucial for accelerating crop improvement (Santos and Leitão 2023; Atkinson and Urwin 2012). Advances in New Breeding Techniques (NBTs), molecular and multi-omics research, and knowledge about previously published QTL, candidate genes, proteins, and metabolites can be applied to understand these intricate processes. Future breeding initiatives may benefit from this and thus can be explored to improve various stress-related traits in leguminous crops. Additionally, these approaches are also applicable for improving traits in microgreens.

Traditional breeding methods, earlier employed for crop improvement, relied on observed phenotypic differences and were thus laborious and time-consuming. However, NBTs allow the quick, reliable, and efficient identification of stress related components. NBTs accelerate the development of novel genotypes with stress related traits for crop breeding programs, resulting in improved nutritional quality and yield under stress conditions (Wani et al. 2016; Hasanuzzaman et al. 2013). Plants's responses to various stresses are governed by their genome; therefore, efforts have been made to understand the stress responses at a molecular level. Legumes have a large genome size, and some exhibit polyploidy, hampering research (Andersen et al. 2005). Model crops, including *Medicago truncatula* and *Lotus japonicus*, among others, have been used to study the genetics behind resistance, potential tolerance, and susceptibility to various stresses. Multi-omics approaches, including genomics, epigenomics, transcriptomics, proteomics, metabolomics, glycomics, inomics, and interactomics, among others, are used for identifying and isolating stress-responsive genes in model legume crops (Ali et al. 2022). Furthermore, genome editing approaches, including RNA interference (RNAi), zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and

CRISPR/Cas, have been used to introduce sequence-specific modifications into the genomes of model and grain legume crops such as alfalfa (*Medicago sativa*), pea (*Pisum sativum*), chickpea (*Cicer arietinum*), soybean (*Glycine max*), peanut (*Arachis hypogaea*), and cowpea (Vigna unguiculata), among others (Détain et al. 2022; Waltz 2018). These technological advancements make significant contributions to developing stress resilient crop varieties. Moreover, they also play a crucial role in enhancing the growth and quality of microgreens derived from leguminous crops.

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2 Abiotic and Biotic Stress in Leguminous Plants

Environmental factors such as salinity, drought, extreme temperature shifts, heavy metal contamination, flooding, exposure to harmful radiation, mechanical and chemical stress, and nutrient deficiency detrimentally impact plant growth and development (Fig. 1, Table 1). The imposition of abiotic stresses not only increases the vulnerability of plants to diseases and pest infestations but also leads to substantial yield loss (Ali et al. 2022; Urva 2021; Latef and Ahmad 2015). Additionally, leguminous crops also encounter a spectrum of biotic diseases instigated chiefly by fungi, nematodes, insects, and weeds, culminating in severe yield detriments (Table 1, Makhumbila et al. 2022; Urva 2021; Sampaio et al. 2020; Nair et al. 2019; Latef and Ahmad 2015; Newton et al. 2011). When exposed to these stressors, legume crops initiate adaptive responses and alter a variety of physiological, molecular, and metabolic pathways, modulating protein and metabolite synthesis, gene expression, and complex signaling cascades. Moreover, as in other plants this stress-induced state often results in an escalated production of free radicals within the legumes (Udawat 2023; Matamoros and Becana 2021; Sita et al. 2017; Araújo et al. 2014). These modifications play a pivotal role in enabling legume crops to withstand and adapt to adverse environmental conditions, thereby influencing the overall sustainability and productivity of agricultural practices (Ali et al. 2022; Urva 2021).

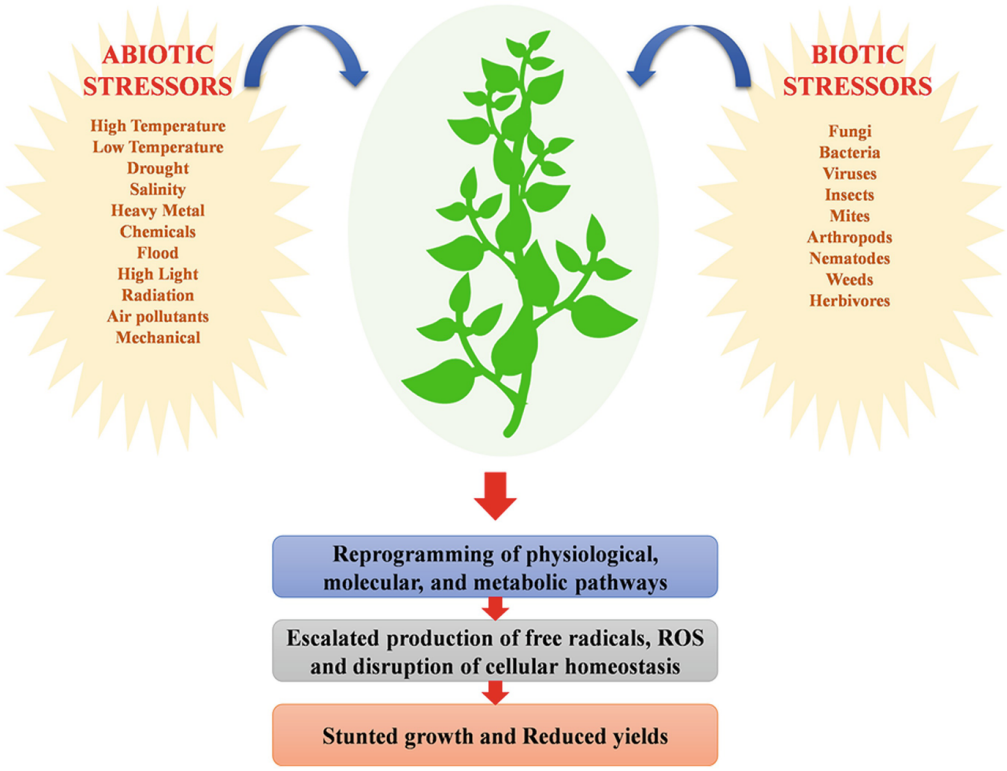


Fig. 1 Legume responses to abiotic and biotic stressors. Abiotic and biotic stress factors pose challenges for legumes, encompassing extreme temperatures, limited light, UV radiation, ozone, saline environments, flooding, drought, heavy metal exposure, acidic or alkaline soil pH, nutrient deficiencies, and the use of agrochemicals like fertilizers and pesticides. Biotic stress arises from insects, herbivores, nematodes, fungi, bacteria, viruses, and weeds. When legume crops confront these stressors, they initiate adaptive responses involving alterations in physiological, molecular, and metabolic processes. These changes encompass shifts in protein and metabolite synthesis, activation of gene expression, initiation of signaling cascades (or transduction pathways), and heightened production of free radicals. Consequently, the modifications in legumes' antioxidant defense systems, alongside alterations in protein and metabolite production, hold heightened significance, given the direct influence of human activities on agricultural productivity

Table 1 List of some of the major stresses affecting legume yield

Legume Crop	Stress condition/causing agent	Growth stages affected/symptoms	Impact at physiological/molecular/biochemical level	References
Cicer arietinum	Drought Stress	Vegetative and reproductive stages	Reduced plant height, relative water content %, chlorophyll index, DTM, secondary branches and leaf protein content, plant yield (33.23% reduction) and filled pods per plant	Sachdeva et al. (2022)
Vigna radita	Salt stress	Seedling stage	Root and hypocotyl length was reduced, increase the contents of phenylpropanoids and flavonoids in mungbean sprouts	Lim et al. (2022)
Medicago sativa	Cold stress	Leaf stage	Reduced, stunted growth, antioxidant enzyme activity in the cold-sensitive WL326GZ genotype	Wang et al. (2022)
Lablab purpureus	Heavy metal	Seeding, leaf and root	Decrease in length, relative water content (RWC), weight of shoot and root, and chlorophyll content	Myrene and Devaraj (2013)
Arachis	Heat Stress	Vegetative and	Reduced chlorophyll index, efficiency of PSII, reduced plant growth, pollen	Spivey et al.

Legume Crop	Stress condition/causing agent	Growth stages affected/symptoms	Impact at physiological/molecular/biochemical level	References
hypogaea		reproductive stages	performance, peg production, and successful pod formation	(2023)
Pisum sativum	Powdery mildew (*Erysiphe pisi*)	Cottony-white fungal colonies and yellowed blotches on the upper and lower surfaces of the leaves	Reduced plant height, seed size, pod setting, disease response, yield	Yimam Assen (2020)
Vicia faba	Chocolate spot disease (*B. fabae, B. cinerea, B. pseudocinerea and B. fabiopsis*)	Lesions (chocolate-coloured spots) on infected leaves, pods and seeds	Reduced plant yield	Bankina et al. (2021)
Phaseolus vulgaris	Common bacterial blight of bean (CBB) (*Xanthomonas citri pv. fuscans* and *Xanthomonas phaseoli pv. phaseoli*)	Water-soaked spots evolving into necrosis on leaves, pustules on pods, and cankers on twigs. In severe infections, defoliation and wilting may occur.	45% yield losses in susceptible genotypes, reduction in photosynthetic tissues.	Chen et al. (2021)
Glycine max	Soybean cyst knot nematode (*Heterodera glycines*)	Yellow dwarf	Reduced yield, grain and seed quality with a negative impact on soybean-derived food, feed, and fuel production	Nissan et al. (2023)
Vigna unguiculata	*Ophiomyia phaseoli*	Swellings at the stem and root junction, causing cracks	Decreased crop yields, stunted growth, failure to produce pods and plant mortality.	Soratur et al. (2018)

Abiotic stress such as heat, drought and cold triggers ROS accumulation, cell damage, membrane and protein degradation, lipid oxidation, and DNA damage, leading to reduced plant growth and development in various legumes including *Glycine max*, *Cicer arietinum*, *Vigna unguiculata*, *Vigna radiata*, *Phaseolus vulgaris*, *Cajanus cajan*, *Vicia faba*, and *Medicago sativa* (Spivey et al. 2023; Wang et al. 2022; Khatun et al. 2021; Nadeem et al. 2019). Additionally, these stresses negatively impact legume grain quality by altering parameters like grain protein content, starch, oil, fatty acid, soluble sugar, and fiber content) in various legumes (Sarkar et al. 2021; Farooq et al. 2018). Legume crops use various mechanisms to counter these stresses. The stress signals perceived by membrane receptors initiate a signal cascade that results in the activation of multiple stress-related genes, including several TFs (DREB, NAC, ABRE, and WRKY) (Khatun et al. 2021; Nadeem et al. 2019). The combination of one or more abiotic stresses, such as drought and heat or drought and cold stress, causes enhanced yield reductions compared to single stress. With the ever-changing climatic conditions, it is anticipated that these stresses will become even more frequent and severe, further impacting plant growth and development in the future (Araújo et al. 2014). Therefore, the goal should be to help legumes survive multiple abiotic stresses by generating climate-resilient legume cultivars with desired traits.

Biotic stressors induce a multitude of symptoms, including stem stunting and wilting, defoliation, root rot, and premature plant death, resulting in significant yield losses (Table 1). Major biotic stresses affecting legume crops are fungal diseases such as chocolate spot disease caused by *Botrytis cinerea*, powdery mildew disease caused by Erysiphe sp., rusts caused by *Uromyces viciae-fabae*, *Uromyces ciceris-arietini*, *Uromyces appendiculatus*, *Uromyces striatus*, *Uromyces pisi*, and *Uromyces vignae*, and wilt disease caused by *Fusarium oxysporum,* which significantly impact both the quality and quantity of legume crops. These diseases can reduce yields significantly impacting overall legume production (Sandhu et al. 2023; Mahmoud 2021). Disease management strategies include the use of fungicides, biocontrol agents, disease free seed material, and crop rotation. However, using genetic resources is the most efficient, cost-effective and environmentally safe method to develop disease-resistant varieties in legumes. In the subsequent section, various approaches to identify and characterize key components of stress response pathways are mentioned. The information deduced from studies on leguminous crops can be effectively utilized to improve microgreens as well. Comparing this data with specific microgreen characteristics can provide valuable insights for optimizing their nutritional content and overall quality.

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3 Approaches to Enhance Stress Tolerance in Legumes

Given the substantial impact of both abiotic and biotic stressors on agricultural production, understanding the intricate interplay between environmental stress and crop responses becomes paramount. The dynamic alterations in physiological, biochemical and molecular processes underscore the need for enhanced research aimed at unraveling the detailed mechanisms involved in the above processes (Santos and Leitão 2023; Atkinson and Urwin 2012). These efforts are crucial for developing innovative strategies to bolster the resilience of crops under stress conditions, optimizing their productivity, and ensuring global food security amidst the challenges posed by human-induced influences on agricultural systems (Sandhu et al. 2023; Ali et al. 2022; Urva 2021). Classical breeding methods aimed at augmenting legume yields have faced efficiency challenges, while conventional approaches have improved genotypes, necessitating extensive time and resources (Majidian 2022; Pratap and Kumar 2011). However, the emergence of next generation sequencing (NGS) based high-throughput genotyping techniques has revolutionized legume research. High-quality genome sequences of many economically important legumes are available (Table 2). The amalgamation of reference genome sequences, high-throughput genotyping technologies, and phenotypic datasets for legume genotypes expedites the efficient identification and monitoring of genetic variations associated with pivotal traits. These advancements, combined with molecular techniques such as marker development, genetic linkage mapping, quantitative trait loci (QTL) identification, expressed sequence tags (ESTs), single nucleotide polymorphisms (SNPs), and integrating transcription factors, expedite precise information delivery to researchers (Udawat 2023; Afzal et al. 2020; Ali et al. 2022). These innovations are poised to accelerate genomics-guided breeding, uncovering novel genes

linked to stress tolerance (Table 3), yield and quality traits, promoting sustainable legume crop production. Enhancing the resilience of legume crops to these stresses might also improve the quality and yield of microgreens. Nutritionally enriched microgreens are developed from good quality seeds, and stress can impact seed quality, thus improving crop resilience ensures a stable supply even in suboptimal environmental conditions. In the following sections, various approaches to improving stress resistance or tolerance in legume crops, along with appropriate examples, are discussed.

Table 2 List of some legumes with sequenced genomes

Legume Species	Sequencing Platform	Genome Size	References
Common Bean (*Phaseolus vulgaris*)	Illumina HiSeq 2000	~532 Mb	Schmutz et al. (2014)
Adzuki Bean (*Vigna angularis*)	Illumina HiSeq 2000	~467 Mb	Kang et al. (2015)
Soybean (*Glycine max*)	Sanger, Illumina	~1.1 Gb	Schmutz et al. (2010)
Lima Bean (*Phaseolus lunatus*)	Illumina HiSeq	~546.4 Mb	Garcia et al. (2021)
Mung Bean (*Vigna radiata ssp.*)	Illumina HiSeq2000	~579 Mb	Kang et al. (2014)
Créole bean (*Vigna glabrescent*)	Illumina HiSeq X Ten (PE150)	~900 Mb	Pootakham et al. (2022)
Peanut (*Arachis hypogaea*)	Illumina HiSeq 2000, PacBio	~2.7 Gb	Bertioli et al. (2019)
Chickpea (*Cicer arietinum*)	Illumina HiSeq 2000	~738 Mb	Varshney et al. (2013b)
Lupin (*Lupinus angustifolius*)	Roche 454, Illumina	~338 Mb	Yang et al. (2013)
Cowpea (*Vigna unguiculata*)	Illumina HiSeq, PacBio	~620 Mb	Muñoz-Amatriaín et al. (2017)
Lotus japonicus	Sanger, Illumina	~470 Mb	Sato et al. (2008)
Pigeonpea (*Cajanus cajan*)	Illumina HiSeq 2000, Roche 454	~834 Mb	Varshney et al. (2011)
Common vetch (*Vicia sativa*)	Illumina HiSeq X Ten	~1.65 Gbp	Xi et al. (2022)
Tepary bean (*Phaseolus acutifolis*)	Illumina X10 and HiSeq 4000	~512.6 Mb	Moghaddam et al. (2021)
White lupin (*Lupinus albus*)	Illumina HiSeq300	~451 Mb	Hufnagel et al. (2020)

Table 3 Studies for improving abiotic and biotic stress resilience in leguminous crops

S.NO.	Approaches	Legumes	Stress Condition/Causing Agent	Study outcome	References
Abiotic stressors					
1	QTL mapping and multi omics	*Glycine max*	Oxidative, salinity and alkalinity	Contrasting seed coat colors (yellow and black) showed similar growth phenotypes but differential tolerance to abiotic stresses	Jiang et al. (2024)
2	Gene expression and biochemical analysis	*Cicer arietinum*	Soil salinity	Salinity affects the phenolic root exudate composition of chickpea plants which further affects the legume-rhizobium interaction. Rhizobial endophytes *Phyllobacterium salinisoli* and *Xanthomonas translucens*	Ben Gaied et al. (2024)

S.NO.	Approaches	Legumes	Stress Condition/Causing Agent	Study outcome	References
				improve the interaction and hence growth under salinity.	
3	Genomic and expression analysis	*Medicago sativa*	Salt and oxidative stress	Plant filamentation temperature-sensitive H (FtsH) proteins, especially *MsFtsH 8* overexpression confers tolerance to salt and oxidative stress, and reduces ROS levels.	Li et al. (2024)
4	QTL-seq, QTL region specific association analysis and molecular haplotyping	*Cicer arietinum*	Heat stress	A functionally relevant gene *CaHSFA5*, with its contrasting haplotypes in tolerant and sensitive chickpea cultivars was identified. The transactivation potential of the said gene was validated which implied its possible role in activating key HSR genes and ROS scavengers to relay HSR in chickpea.	Mohanty et al. (2024)
5	Biochemical and expression analysis	*Cicer arietinum*	Drought stress	Drought tolerant genotypes indicated increased expression of RCI2 genes compared to the sensitive ones. Additionally, tolerant genotypes could increase the cell membrane stability and moderate ROS content by improving the amount of proline and antioxidant enzymes.	Forghani and Aghaie (2024)
6	Genomic and expression analysis	*Medicago sativa*	Multiple abiotic stresses (drought, salt, ABA and cold)	The study highlights beta-glucosidases (BGLUs) play crucial roles in response to various stresses and hormonal responses, hence are candidates for stress tolerance breeding in alfalfa.	Kong et al. (2024)
7	Epigenetics (DNA methylation)	*Medicago sativa*	Recurrent drought stress	Recurrent drought stress decreases the total DNA methylation levels when compared to a single drought stress.	Ventouris et al. (2020)
8	Biological, physiological and biochemical analysis.	*Phaseolus vulgaris*	Heavy metals	HMs decreased the photosynthetic pigments and leaf greenness. Additionally, the highest adverse effects were observed with Cd treatments as compared to Pb and Ni.	Hammami et al. (2022)
9	Physiological and biochemical analysis	*Cicer arietinum*	Cold stress	Cold tolerance in chickpea relates to the better ability of anthers and ovules to acclimate, involving various antioxidants and cryoprotective solutes.	Rani et al. (2021)
10	Transgenesis	*Cicer arietinum*	Water deficit	Stress inducible expression of *AtDREB1a* gene in transgenic chickpea lines greatly enhanced drought tolerance when severe drought stress was imposed.	Das et al. (2021)
Biotic stressors					

S.NO.	Approaches	Legumes	Stress Condition/Causing Agent	Study outcome	References
1	GWAS and comparative genomics	*Lathyrus sativus*	Powdery mildews (*Erysiphe pisi* and *Erysiphe trifolii*)	Analysis of 189 global grass pea accessions revealed distinct responses, suggesting independent genetic basis for resistance to the two pathogens. The findings emphasize the importance of complementary breeding approaches to introduce resistance to both powdery mildews in grass pea varieties, with identified resistance sources and genomic targets aiding in this breeding effort.	Martins et al. (2023)
2	RNA-seq and proteomics	*Pisum sativum*	Root rot disease (*Aphanomyces euteiches*)	Through genome mining and expression analysis, tandemly repeated proteases, especially those upregulated during host infection, were identified. Using Activity Based Protein Profiling and mass spectrometry, 35 active extracellular microbial proteases were discovered, suggesting their potential role as pathogenicity factors in the Aphanomyces genus.	Kiselev et al. (2023)
3	GWAS	*Lens culinaris*	Stemphylium blight (*Stemphylium botryosum*)	Four QTLs on chromosomes 2, 4, and 5 were identified, along with five significant single nucleotide polymorphism (SNP) markers within these QTL regions and seven markers on other chromosomes, providing potential tools for marker-assisted selection in developing stemphylium blight-resistant lentil cultivars.	Adobor et al. (2022)
4	LC-QqQ-MS	*Glycine max*	Fungal stress (*Aspergillus oryzae* and *Rhizopus oligosporus*)	A unique isoflavone O-methyltransferase (IOMT), named GmIOMT1, in soybean, catalyzing the final step of glycitein biosynthesis. Multi-omics analyses revealed that glycitein production was induced in response to fungal stress, and overexpression of GmIOMT1 increased glycitein levels in soybean roots, suggesting its role in biotic stress-induced glycitein biosynthesis.	Uchida et al. (2020)
5	Transcriptome and Metabolome	*Medicago sativa*	Thrips infestation (*Thysanoptera spp.*)	In alfalfa, thrips infestation triggers significant changes in flavonoid and isoflavonoid biosynthesis pathways. The study highlights the crucial role of SA and JA signal transduction, PAPM-triggered immunity, and the MAPK signaling pathway in thrips-induced plant resistance,	Zhang et al. (2021)

S.NO.	Approaches	Legumes	Stress Condition/Causing Agent	Study outcome	References
				providing insights into herbivore-induced plant defense mechanisms and aiding in the development of strategies against thrips in alfalfa.	
6	Comparative RNA-Seq	*Vigna radiata*	Yellow mosaic disease (*Mungbean Yellow Mosaic India Virus*)	A complex gene network involving PAMPs, signaling cascades (kinases, jasmonic acid, brassinosteroid), and specific genes (PR-proteins, virus resistance, R-gene proteins), key TFs and proteins leading to resistance against Mungbean Yellow Mosaic India Virus (MYMIV) were identified.	Dasgupta et al. (2021)
7	Proteome and metabolome	*Cicer arietinum*	Fusarium infection (*Fusarium oxysporum*)	The study identified 325 chitosan-triggered immune-responsive proteins and 65 metabolites, revealing a comprehensive network involving ROS, NO, eATP, and oligosaccharide signaling, contributing to Fusarium resistance and highlighting ECM and guard cell remodeling in the chitosan-triggered immunity pathway.	Narula et al. (2020)
8	GWAS and Genomic Prediction	*Phaseolus vulgaris*	Root rot (*Pythium myriotylum*)	While no major resistance genes were identified through GWAS, genomic prediction proved effective for Pythium, plant vigor, and related traits, suggesting a quantitative resistance mechanism, and indicating the potential of this method for estimating tolerance to root rot in common beans.	Diaz et al. (2021)
9	Whole Genome Data	*Vigna radiata*, and *Glycine max*	Tan Spot (*Curtobacterium flaccumfaciens pv. flaccumfaciens*)	High-quality genome assemblies of *Curtobacterium flaccumfaciens pv. flaccumfaciens* strains linked to mungbean and soybean, revealing plasmid variability and carbohydrate-active enzymes, provide insights into the pathogen's genetic basis, host association, and adaptation.	Vaghefi et al. (2021)
10	Transcriptome	*Glycine max*	Charcoal rot (*Macrophomina phaseolina*)	Differentially expressed genes revealed the activation of signaling pathways, including calcium and WRKY-genes, in the resistant genotype, suggesting a quantitative resistance trait. Genes such as WRKY, LysM-RLKs, NBS-LRR, and flavonoid pathway genes were identified as potential candidates for developing genetic resistance	Deshmukh and Tiwari (2021)

S.NO.	Approaches	Legumes	Stress Condition/Causing Agent	Study outcome	References
				against charcoal rot in soybean breeding programs.	

3.1 Genetic Resources

Legume genetic resources are a source of genetic diversity and starting material for the development of varieties with improved grain and forage quality, improved tolerance to abiotic stresses, major diseases, and pests (Miladinović et al. 2021). Several genetic resources and databases related to legume genetic resources have been developed (Table 4). International organizations, research centers, and countries officially collaborated in the year 1971 to form the Consultative Group on International Agricultural Research on Resources (CGIAR) under World Bank supervision. Further collaboration with the Food and Agriculture Organization (FAO) led to the establishment of the International Board for Plant Genetic Resources (IBPGR) in 1974. Genebanks hold thousands of accessions of varieties and wild species, as well as important breeding lines. To facilitate accessibility and better use of the germplasm available with gene banks (CIAT, IITA, AVDRC, and CGIAR), various sets of core, mini-core, focused identification of germplasm strategy (FIGS), and reference germplasm have been developed (Odong et al. 2011; Mackay et al. 2004; Upadhyaya and Ortiz 2001; Brown 1989; Frankel 1984).

Table 4 List of some legume databases and genetic resources

Name of the databases	Website
The second report on the state of the world's plant genetic resources for food and agriculture	https://www.fao.org/3/i1500e/i1500e00.htm
GRIN	https://www.ars-grin.gov/
Genesys	https://www.genesys-pgr.org/
International Center for Tropical Agriculture (CIAT)	https://www.ifpri.org/donor/international-center-tropical-agriculture-ciat
International Institute of Tropical Agriculture (IITA)	https://www.iita.org/
World Vegetable Center (AVRDC)	https://avrdc.org/
Institute of Field and Vegetable Crops	https://ifvcns.rs/en/home-page/
Consultative Group on International Agricultural Research	http://www.cgiar.org/
Legume Information System (LIS)	https://legumeinfo.org/
International Crops Research Institute for the Semi-Arid Tropics	http://www.icrisat.org/
Kazusa DNA Research Institute for the *Lotus japonicus* Genome	http://www.kazusa.or.jp/lotus/
European Medicago Consortium	https://medicago.toulouse.inra.fr//
LegumeIP	https://www.zhaolab.org/LegumeIP/gdp/
PeanutBase	https://www.peanutbase.org/
Integrated Chickpea Transcriptome Database	http://nipgr.res.in/ctdb.html.
LegumeInfo QTL Database	https://www.legumeinfo.org/
Pulse Crop Database	https://www.pulsedb.org/main
Lotus Base	https://lotus.au.dk/

3.2 Conventional Breeding

Conducting pre-breeding activities such as phenotypic and genotypic assessment of germplasm collections is essential to a breeding program, which not only gathers fundamental information about the genetic relations among accessions but also suitable parents for cross-breeding. Legume germplasm banks like, International Center for Tropical Agriculture (CIAT), the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), the International Center for Agricultural Research in the Dry Areas (ICARDA), the International Institute of Tropical Agriculture (IITA), and the Australian Temperate Field Crops Collection (ATFCC) serve as reservoirs of genetic diversity and are crucial for legume breeding. Since most of the legumes are autogamous with low outcrossing rate values, they display moderate variability at intragroup levels (Pratap and Kumar 2011). Contrarily, legume germplasms display diverse phenological traits with respect to hypocotyl color, days to flowering, flower number, pod color, and seed weight (Okii et al. 2017). Conventional breeding has been successful in improving monogenic traits like shape, texture, and color; however, polygenic quantitative traits, which are affected both by the environment and genotype x environment require advanced breeding methodologies (Kumar and Ali 2006). Conventionally, the effectiveness of legume breeding programs hinged on the thorough phenotypic and genetic evaluation of germplasm, aiming to identify superior parental lines (Fig. 2).

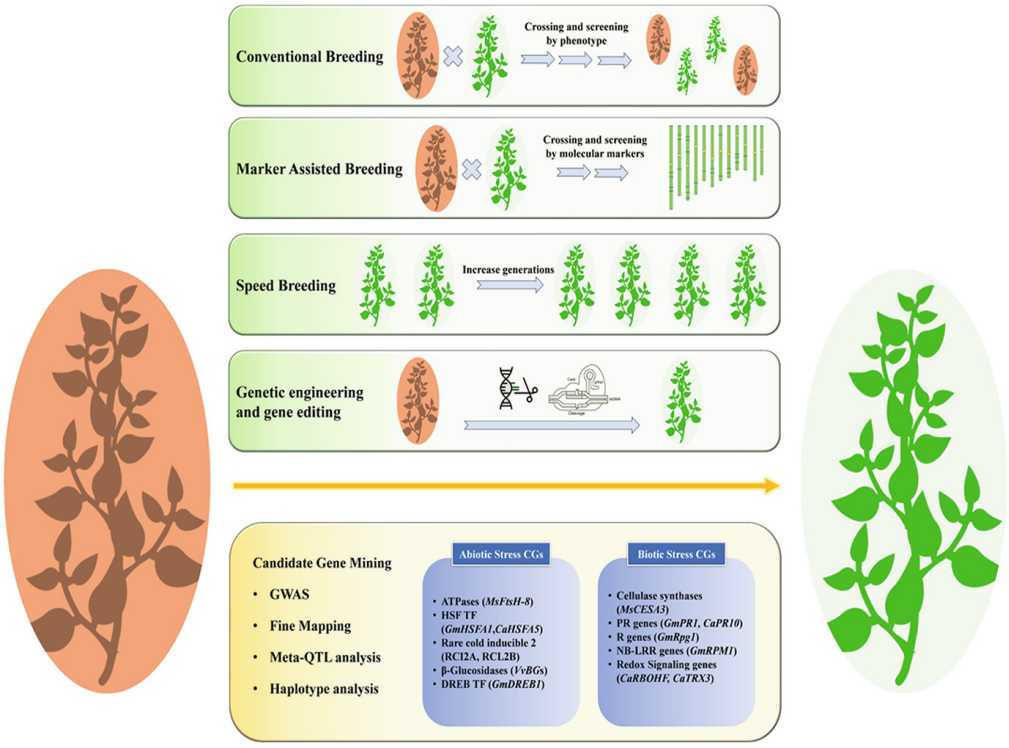


Fig. 2 Comparison of various breeding strategies in legumes, CG mining techniques and CGs that can be utilized for abiotic and biotic stress tolerance through modern breeding. Conventionally, mapped populations were selected on the basis of phenotypic superiority. The advent of MAB using molecular markers made the screening process simpler and streamlined. Additionally, speed breeding has drastically reduced the time to generate plants with desired phenotype. The more recent gene editing technology has eased the process of generating genetically elite varieties. Further, candidate gene mining techniques like Genome Wide

Association Mapping (GWAS), fine mapping of major QTLs, meta analysis of all previously known QTLs and haplotype analysis of associated regions can accelerate the pace of breeding strategies

3.3 Multi-omics Approaches for Understanding Stress Tolerance Mechanisms in Legumes

Modern legume breeding is an amalgamation of QTL mapping through marker assisted selection (MAS), marker-assisted backcrossing (MABC), multiparent advanced generation inter-crossing (MAGIC), marker-assisted pyramiding and identification of genomic regions under selection (Varshney et al. 2013a). The accelerated single seed descent method, which shortens generation cycle by promoting in vitro flower and fruit set has been a notable advancement that has enhanced the speed of breeding (Mobini et al. 2014). Additionally, the boom in NGS technologies has led to the unprecedented development of multi-omic methods such as genomics, transcriptomics, proteomics, and metabolomics (Naqvi et al. 2024; Gupta et al. 2023; Ali et al. 2022). These multi-omics approaches encompass a thorough and simultaneous analysis of various facets of biological data. This includes in-depth analysis at the molecular, genomic, proteomic, metabolomic, phenomic, and ionomic levels, which help in getting detailed information and understanding of potential candidates, signaling pathways, and processes responsible for regulating stress tolerance in leguminous plants (Fig. 3). By gaining insights at multiple levels, researchers can validate identified key genes, proteins, and metabolites, leveraging this information for enhancing stress tolerance in legumes, which is crucial for generating stress tolerant varieties and cultivars. In the ensuing section we discuss in depth about these omic approaches.

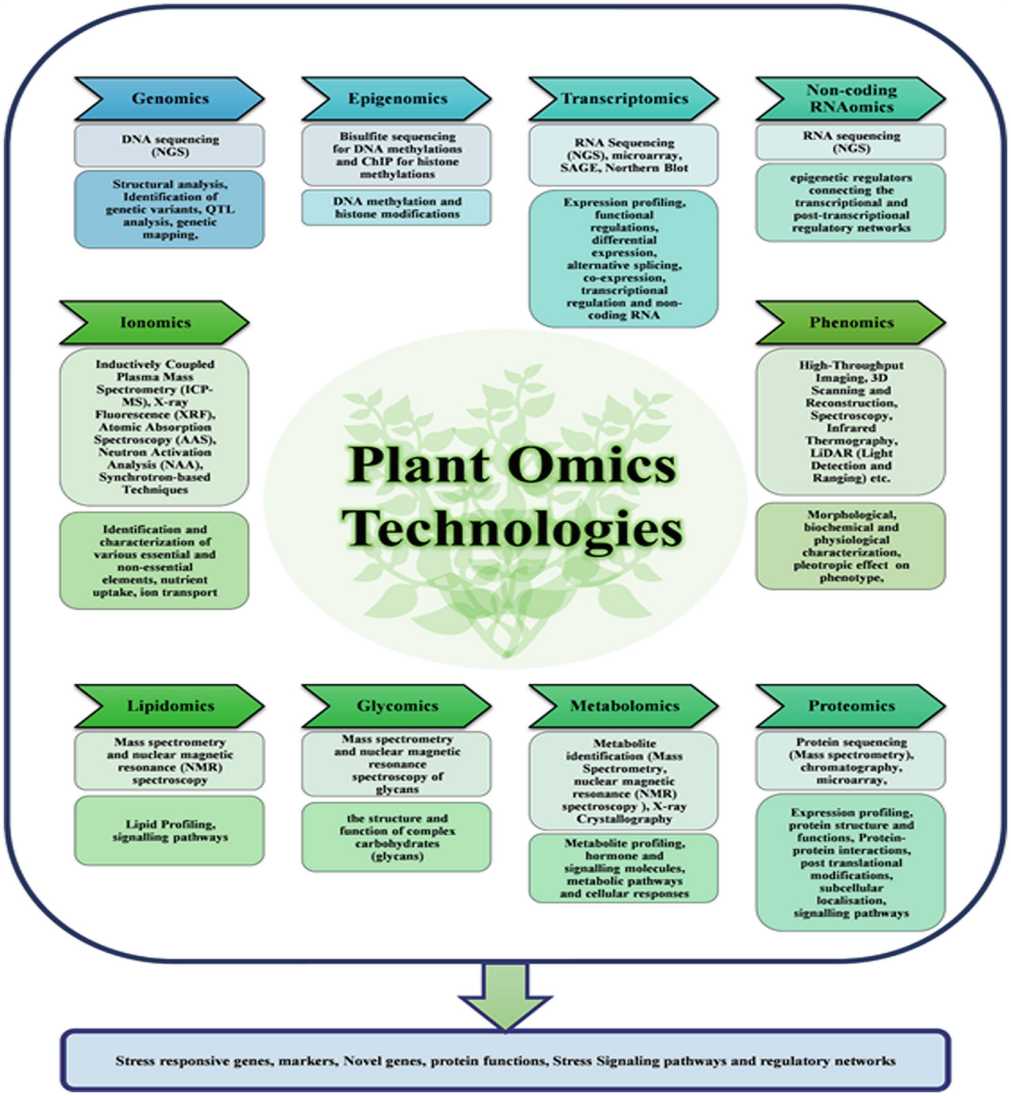


Fig. 3 Illustration depicting the holistic landscape of omics approaches. These comprehensive tools play pivotal roles in unraveling the intricate molecular details underlying biological systems, enabling a holistic understanding of complex scientific phenomena

3.3.1 Genomics

Genomics is the study of complete sets of DNA, focusing on the structure, function, and evolution of an organism's genome. It helps researchers identify genetic variations, including single nucleotide polymorphisms (SNPs), insertions/deletions (Indels), chromosomal rearrangements, and other structural variations. Legume crops exhibit variations in their ploidy levels, ranging from diploid to polyploid; this variation in ploidy contributes to the variable genome size and genetic diversity, which affects genomic research, evolutionary analysis, and breeding strategies. To better recognize legume biology, basic research on legumes is performed on the model systems *Lotus japonicus* and *Medicago truncatula*, because of their diploid small genome, short life cycle, and enhanced seed production (Cook 1999; Cervantes et al. 2012). Furthermore, the genome sequence of various legume crops, including the model legumes *Lotus japonicus* and *Medicago truncatula,* is now available. This includes *Glycine max*, *Phaseolus*

lunatus, *Cajanus cajan*, *Vigna radiata ssp.*, *Cicer arietinum*, and many additional species (Table 2). These genomic resources help in identifying and characterizing agronomically important and stress responsive genes. They can also identify genetic pathways underlying diverse traits and act as reference maps for marker development. Some genome assisted crop improvement approaches are marker-assisted selection (MAS), genomic selection (GS), association mapping (AM), genome editing, genome-wide association studies (GWAS), and comparative genomics. These genome approaches are extremely valuable, and although they serve as a great initial point, they are insufficient to understand both gene activity and the metabolic and regulatory responses that get stimulated as the stress condition prolongs. These barriers can be overcome by studying stress responses at transcriptomic, proteomic, and metabolomic levels using advanced techniques (Dita et al. 2006), which are discussed below.

3.3.1.1 Molecular Markers and Genomic Resources

Genetic markers are nucleotide sequences with a known chromosome location controlling a particular trait. They are closely related with the target gene, acting as signs or flags. Classical markers such as morphological, cytological and biochemical along with DNA or molecular markers such as restriction fragment length polymorphism (RFLP), simple sequence repeats (SSRs), randomly amplified polymorphic DNA (RAPD) are important developments in the field of plant breeding (Nadeem et al. 2017). However, the advent of the sequencing technologies like NGS and genotyping by sequencing (GBS) have revolutionized plant breeding through development of SNPs resulting in high polymorphism. Legume crops use three types of genotyping technology platforms: high density (> 20 K SNPs), mid-density (2–5 K SNPs), and low density (>100 SNPs) for detailed genetic analysis and breeding purposes. High-density genotyping performed using NGS technologies (such as Illumina, PacBio), and GBS enables trait mapping using genome-wide association studies (GWAS), leading to the identification of genes associated with specific traits. Mid-density genotyping requires AgriSeq, DartTag, and RiCA platforms for mapping populations for trait mapping via genetic mapping and genomic selection (GS). Lastly, low-density genotyping uses TaqMan and Kompetitive Allele-Specific Polymerase Chain Reaction (KASP™) for performing early generation screening, MAS, quality control (QC), and hybrid purity testing (Semalaiyappan et al. 2023; Varshney et al. 2018a). These approaches are fast and time-efficient and enable trait mapping by discovering genomic regions and candidate genes for downstream applications such as gene cloning and molecular breeding research. Thus the amalgamation of the genomic resources and MAS contribute to identification of specific genes or regions associated with traits, open new possibilities for genetic improvement of complex traits and accelerate the breeding process (Varshney et al. 2018a; Nadeem et al. 2017; Saade et al. 2016). SNPs are ideal for discerning complex traits by employing multiplexed marker oligonucleotides such as the Affymetrix GeneChip, which is efficient, co-dominant, high-throughput, cost-effective, highly abundant, and sequence-tagged (Missanga et al. 2021; Thudi et al. 2021). Furthermore, high throughput Axiom® SNP array genotyping is also a fast and inexpensive method for genotyping and the development of high-density linkage maps. The Axiom® SNP Arrays with more than 50 K SNPs have been developed in

many legumes including pigeonpea (Saxena et al. 2018), chickpea (Roorkiwal et al. 2017) and groundnut (Pandey et al. 2017).

3.3.1.2 Quantitative Trait Loci (QTL) Mapping

The heredity of stress resistance is a complex phenomenon, and QTL mapping is the most popular method of discovering QTL, as well as genetic and linkage mapping of genomic regions related to tolerance (Kushwah et al. 2021). Linkage maps are crucial for understanding genetic linkage and distances between markers on a chromosome and often incorporate sequence-tagged markers like SNPs, AFLPs, or SSRs. These markers act as anchor points, facilitating spatial replication of significant genes and providing valuable insights for functional genomics and evolutionary studies. Markers are effective in determining the QTLs associated with specific traits, as well as for identifying genotypes resilient to different abiotic and biotic stresses and sometimes exhibiting multi-stress tolerance. Recent advancements in genomics and phenomics have enabled better characterization of the QTLs associated with desired traits, collectively referred to as QTLomes (Salvi and Tuberosa 2015). Furthermore, meta-QTL analysis, QTL impact estimation, and crop modeling will enable effective use of QTLome against various biotic and abiotic stresses in legumes. Several studies have been conducted to dissect QTLs in legume crops for abiotic and biotic stresses (Table 3, Luo et al. 2023; Diaz et al. 2018; Wang et al. 2019; Bhadauria et al. 2017; Rehman et al. 2011; Muchero et al. 2009). Genomic regions harboring stress associated QTLs governing salinity tolerance were identified in chickpea, including CaLG01 (salsBqtl.2, salWUEqtl.1), CaLG05 (salClqtl.1, saltolSYqtl.1), CaLG04 (salFPqtl.1, salNecrosisqtl.2, salSNqtl.1, salSY.2, saltolWUqtl.1; saltolFPqtl.1; saltolSNqtl.1; saltolSYqtl.2), and CaLG06 (saltolSNqtl.2). Positional cloning of these salinity tolerance-specific loci will further improve our understanding of the key determinants of salinity tolerance in chickpea (Atieno et al. 2021). Three QTLs viz. Fsp-Ps 2Fsp-Ps3.2 and Fsp-Ps3.3 associated with Fusarium root rot resistance were identified in *Pisum sativum* (Coyne et al. 2019). Specific QTLs for microgreen-related traits, such as shelf life and nutrient content (Fe, Zn), have been identified in chickpea and other microgreens using contrasting parental lines and genomic data (Mahto et al. 2022).

3.3.1.3 Genome-Wide Association Studies (GWAS)

GWAS proves valuable in overcoming limitations of traditional gene mapping by offering higher resolution, often at the gene level. Additionally, GWAS utilizes samples from previously well-studied populations, enabling the association of commonly occurring genetic variations with phenotypic variation (Breria et al. 2020). GWAS requires structuring the population into diverse panels to estimate genetic diversity and minimize the detection of false positives and minor allele QTL influences. Recent advancements in NGS applications and new breeding techniques (NBTs) have revolutionized the ability to characterize genetic variations at a high resolution. These technological innovations provide researchers and breeders with the ability to develop new stress resilient varieties, contributing significantly to crop improvement (Liu et al. 2022; Gali et al. 2019). Association mapping/GWAS is gaining popularity in legume crop improvement because of its abilities to discover QTLs with precision without using additional resources for population growth. GWAS has made it possible to access functional

genetic variations for abiotic stress tolerance and biotic stress resistance traits in legume germplasms with greater accuracy and allelic richness (Table 3, Susmitha et al. 2023). In soybean, GWAS using 34,718 SNPs in a panel of 243 global soybean collections identified the major SNPs, Gm_08_11971416, Gm_08_46239716, Gm_05_1000479, and Gm_01_53535790, associated with seed flooding tolerance related traits, viz. electrical conductivity, germination rate, shoot length, and root length (Sharmin et al. 2021).

3.3.2 Epigenomics

Epigenomics explores DNA methylation and post translational histone modifications modifications (acetylation, methylation, phosphorylation, and ubiquitination) that influence gene expression without altering the underlying DNA sequence. Genome-wide profiles of epigenetic changes in legumes, facilitated by NGS, reveal widespread alterations in response to stresses, highlighting the importance of analyzing specific modified genes and represented pathways for a comprehensive understanding of stress tolerance variations among legume species (Yung et al. 2022; Zhao et al. 2022). By targeting these epigenetic mechanisms, researchers aim to develop legume varieties with improved responses to abiotic stresses and enhanced resistance to diseases (Yung et al. 2022; Chandana et al. 2022). High-coverage methylomes for soybean and common bean reference genomes have been generated, offering valuable resources for analyzing epigenetic variation (Kim et al. 2015). In soybean, under heat stress, differentially methylated regions (DMRs) in single-cell root hairs and multicellular stripped roots were identified. Hypomethylation was observed in both single-cell root hairs and multicellular stripped roots in all contexts, especially in the CHH context. Moreover, mRNA expression analysis identified associations between DMRs, genes, and transposons (Hossain et al. 2017). Specific methylation QTLs (meQTLs) have been identified for traits desirable in microgreens, such as shelf life, iron and zinc content (Kumar et al. 2016).

3.3.3 Transcriptomics

Transcriptomics is an approach for quantifying gene expression, identifying the gene regulatory pathway and candidate genes (CGs) responsible for stress resilience in legumes, and helping in crop breeding (Afzal et al. 2020). Serial analysis of gene expression (SAGE) and microarrays can be used to determine extensive transcriptome information due to advanced high-throughput analysis. Differentially expressed genes (DEGs) are usually evaluated by sequencing the ribo-nucleic acid pool in the cell at a particular time point (microarray, RNA-seq). Another technique that is used for identification, quantification of gene expression, and annotation is digital gene expression (DGE) (Yu et al. 2019). Transcriptome analysis, aiming to identify differentially expressed genes in response to various abiotic (water deficit, drought, cold stress, and salt stress) and biotic (Fusarium wilt) stresses in common beans and other leguminous crops, has been reported (Zhang et al. 2023; Yang et al. 2023; Subramani et al. 2023; Leitão et al. 2021, Table 3). For instance, comparative RNA-Seq analysis in *Vigna radiata* identified several stress specific genes, such as PR-proteins, R-gene proteins, key TFs, and proteins that could provide resistance against the Mungbean Yellow Mosaic India Virus (MYMIV) (Dasgupta et al. 2021). Thus, future breeding initiatives may benefit from the identification of candidate stress

responsive genes (Dai et al. 2020). Furthermore, the identified DEGs can be converted into molecular markers and utilized in breeding programs for the production of stress-tolerant leguminous crops and future microgreens.

3.3.4 Non-coding RNA Omics

Noncoding RNAs (ncRNAs), which include microRNAs (miRNAs), small interfering RNAs (siRNAs), long noncoding RNAs (lncRNAs), and circular RNA (circRNA), regulate gene expression at several stages, including transcription, post-transcription, and translation. These ncRNAs interact with DNA, RNA, and proteins to control the activity of their target genes, modulating plant growth, development, and stress responses (Zhang et al. 2019). Furthermore, studying the regulation of these ncRNAs, as well as housekeeping ncRNAs such as circRNAs, small nucleolar RNAs (snoRNAs), rRNAs, and tRNAs, may provide insights that lead to the emergence of innovative methods for increasing legume crop production and resilience (Chand Jha et al. 2021). Root small RNA transcriptomes in *Medicago truncatula*, the model legume, were analyzed under symbiotic and pathogenic interactions, yielding 416 miRNA candidates, 52 of which were novel miRNAs. These miRNAs play a crucial role in controlling root development adaptation to the soil environment in *M. truncatula*, including responses to bacterial and fungal pathogens, symbionts, and related Nod and Myc-LCO signals (Formey et al. 2014). A genome-wide analysis of the peanut transcriptome revealed 1442 lncRNAs, mostly from intergenic regions, including 189 lncRNAs differentially expressed in roots, leaves, or seeds. Predicted target genes of these lncRNAs were found to be associated with diverse biological processes and stress response pathways. Selected lncRNAs and their target genes demonstrated varied responses to salt stress, suggesting their potential role in influencing salt stress responses (Tian et al. 2020).

3.3.5 Proteomics

Proteins play roles in cell signaling pathways, stress adaptation, repair, and other host plant functions, aiding in stress tolerance, recovery and improving survival in challenging situations (Hakeem et al. 2012). Analyzing proteome composition and developmental changes, including stress responses, can improve stress resilience in leguminous crops and also the quality and shelf life of microgreens (Gupta et al. 2023). Recent advancements in proteomic studies, employing techniques like mass spectrometry (MS) and various software tools, enable precise and comprehensive protein quantification. Methods such as two-dimensional gel electrophoresis, shotgun proteomics, selected reaction monitoring (SRM), and protein-protein interaction (PPI) analyses contribute to understanding molecular mechanisms under stress conditions in legumes (Chandramouli and Qian 2009). Proteomic analysis has facilitated the identification of differentially expressed proteins in response to various biotic and abiotic stressors in leguminous crops, including soybean, peas, chickpea, and common bean (Table 3, Subramani et al. 2024; Kiselev et al. 2023; Narula et al. 2020; Vessal et al. 2020). The proteins induced in response to these stresses are largely common, belonging to overlapping metabolic pathways involved in photosynthesis, signal transduction, protein metabolism, antioxidant, and cell defense mechanisms (Jan et al. 2022). A comparative proteomics study in chickpea root revealed 75 differentially expressed proteins (DEPs) under drought stress. Proteins involved in carbon and

energy metabolism; stress response; protein metabolism, ROS metabolism, signal transduction, secondary metabolism, nitrogen (N), and amino acid metabolism. The identified proteins can be used for genetic improvement programs to develop drought-tolerant chickpea lines (Gupta et al. 2020).

3.3.6 Metabolomics and Glycomics

Metabolites, in contrast to mRNA or proteins, depict transcriptional interactions regulating gene expression during adverse conditions that could be linked to the phenotype (Wienkoop et al. 2008). Metabolomic studies, employing various techniques like gas chromatography–mass spectrometry (GC–MS) and capillary electrophoresis–mass spectrometry (CEMS), enable the identification of stress-related components and provide insights into plant responses under different conditions (Larrainzar et al. 2009). Metabolomics has been applied in studies on symbiotic interactions in Medicago, salinity and drought stress in Lotus, flooding stress in soybean roots, and phosphorus-deficiency stress in common bean roots, offering valuable insights into metabolic responses under diverse stressors (Ramalingam et al. 2015; Staudinger et al. 2012; Sanchez et al. 2011; Komatsu et al. 2011; Hernández et al. 2009). A comparative metabolic study in *Vicia faba* determined the ion-specific effects of Cl−, Na+, and K+ on primary metabolite pools. Allantoin, myo-inositol, and glycerol phosphoglycerol levels were significantly upregulated at high salt concentrations, in the roots of both cultivars (Richter et al. 2018). Furthermore, studying metabolome variations can also improve microgreen traits.

The glycome represents the entire collection of glycans, their modifications, either independently or conjugated to noncarbohydrate biomolecules like proteins, lipids, and nucleic acids. Glycomics aims for a comprehensive investigation into the structural and functional aspects of the glycome, uncovering its dynamic spatiotemporal changes intricately linked with diverse biological processes (Gupta et al. 2010; Trbojević-Akmačić et al. 2022). In a glycomic investigation, the N-glycan structures of Lotus seed globulins were comprehensively analyzed through mass spectrometry-based glycomics and glycoproteomics techniques, identifying Lotus convicilin storage protein 2 (LCP2) as a major food allergen (Dam et al. 2013). Furthermore, plants rely heavily on glycans for cell wall integrity, signaling, communication, and pathogen interactions. Studying their changes under stress conditions might aid in elucidating the impact of these processes on stress tolerance.

3.3.7 Lipidomics and Ionomics

Lipidomics is an in-depth study of the complex processes and networks that regulate cellular lipids, studied by employing mass spectrometry, chromatography, and spectroscopy (Yang and Han 2016). It explores the composition, structure, and functions of lipids, as well as their regulation and interactions with other molecules within legume crops. By understanding lipid metabolism in legume crops, researchers can develop strategies to improve crop productivity, nutrient content, and stress tolerance (Zhang et al. 2022a, b; Narayanan et al. 2020; Okazaki et al. 2017). A comprehensive transcriptomic and lipidomic analysis of cold-tolerant (NH5) and cold-sensitive (FH18) peanut varieties uncovered the pivotal role of lipid metabolism, particularly membrane lipids and fatty acids, in conferring cold tolerance (Zhang et al. 2020, 2022b).

Heat-induced lipidomic changes in soybean heat-tolerant DS25-1 genotype and heat-sensitive DT97-429 genotype, revealed a decline in lipid unsaturation, particularly linolenic acid (18:3), in the heat-tolerant DS25-1 genotype. This reduction was associated with reduced expression of FAD3A and FAD3B genes, highlighting a potential mechanism of maintenance of membrane functionality and heat tolerance in DS25-1 genotype (Narayanan et al. 2020).

Ionomics, assessing elemental components in response to physiological functions, is vital for understanding an organism's metal, nonmetal, and metalloid composition (Huang and Salt 2016; Salt et al. 2008). The term "ionome" evolved into "metallome" to encompass ecologically essential non-metals like nitrogen, sulfur, and phosphorus (Salt et al. 2008). Ions maintain plant homeostasis under various environmental situations, with ion transporters crucial for metabolic processes and stress management. Ionome evidence aids experiments on micronutrient accessibility in legumes, requiring a thorough understanding of transcriptional regulation for assessing stress-responsive ion transporters, genetic mutations, and other constituents. Integrating ionomics with other pan-omics tools expands possibilities for evaluating abiotic and biotic stress impacts in legumes, enhancing legume productivity (Singh et al. 2021; Niron et al. 2020).

3.3.8 Interactomics

Interactomics involves the study of complex molecular interactions including protein-protein, protein-DNA, protein-RNA, protein-lipid, and protein-metabolite interactions (Fig. 4) (Li et al. 2018). Understanding these diverse interactions is crucial for unraveling cellular processes and signaling pathways. Various experimental and computational methods are employed to comprehensively analyze the intricate web of biomolecular relationships. Interactomics study on *Phomopsis longicolla* revealed 1414 pathogenicity-related genes and 149 plant cell wall degrading enzymes (PCWDE), offering valuable insights into pathogenicity mechanisms. This study showcases the potential of interactome studies in understanding soybean pathogen interactions (Li et al. 2018). The PPIs are usually validated with techniques such as pull-down assays, yeast two hybrid assay, co-immunoprecipitation, in-situ hybridization.

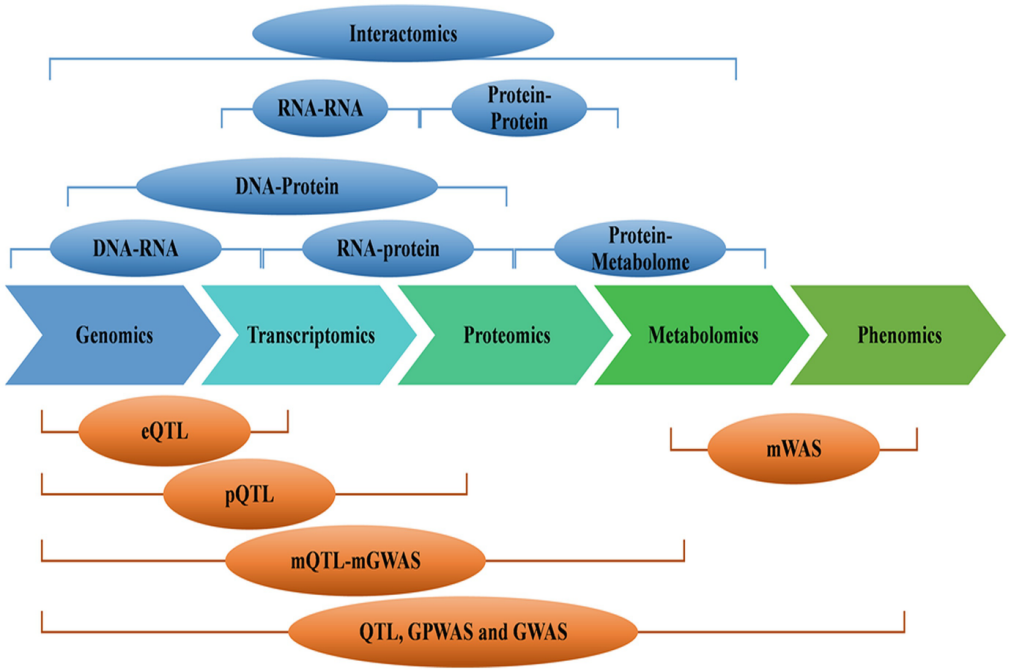


Fig. 4 Integration of Multi-Omics Technologies and QTL Studies. The omics layers include genomics, transcriptomics, proteomics, metabolomics, and phenomics. The interaction of multi-omics studies involves interactions among DNA, RNA, proteins, and metabolites (DNA-RNA, DNA-protein, RNA-RNA, RNA-protein, protein-protein, and protein-metabolome). These interactions are essential for understanding cellular functions and regulatory mechanisms. Integration of omics data in QTL studies, such as expression QTL (eQTL), protein QTL (pQTL), metabolite QTL (mQTL), metabolome-wide association studies (mGWAS), genome-wide association studies (GWAS), genome-phenome-wide association studies (GPWAS), and metabolome-wide association studies (mWAS), identifies genomic loci associated with variations in gene expression, protein levels, metabolite levels, and phenotypic traits

3.3.9 Phenomics

Phenomics isa research front that analyzes plant phenotypes on a large scale. Phenotypes result from a mix of genetics and the environment. Precise phenotyping is crucial to understand biological processes (Zhao et al. 2019). By analyzing traits such as plant height, root length, leaf number, and responses to stressors like wilting, chlorosis, necrosis, and lesions, phenomics provides valuable insights into the performance and resilience of legumes (Zhao et al. 2019; Burridge et al. 2020; Rane et al. 2021). Genetic markers are assessed using advanced technologies for phenotype-based evaluations, and the connection between genetic markers and phenotype consistency determines genomics' effectiveness (Varshney et al. 2018b). In plant breeding, omics approaches, particularly phenomics, hold promise for enhancing genetics to achieve desired phenotypes for improved yield under diverse environmental conditions (Biswas et al. 2021). Abiotic stress tolerance and biotic stress resistance are critical for yield persistence, and phenotyping aids in evaluating traits related to stress tolerance in legumes under controlled conditions at sowing (Munns 2010). Phenotyping allows real-time imaging of characters of interest, and Near-Infrared Spectroscopy (NIRS) is a dynamic technique in advanced phenotyping studies for plant breeding. Using the high throughput phenotyping (HTP) system, 60 accessions of chickpea were evaluated for growth, physiology, and recovery traits under drought stress conditions. For more than 40 days, multiple traits, including

estimated biovolume, plant height, and several color and photosynthesis related traits, were examined in contrasting water regimes. The study identified that loss of estimated biovolume, photosynthetic efficiency, and change in color can be used as indicators of different stages of drought stress, as these traits are associated with drought stress progression (Lauterberg et al. 2023).

3.4 Genetic Engineering (GE)

Genetic engineering involves the direct manipulation of an organism's genes using biotechnological methodologies to achieve desired traits or characteristics. Genetic transformation (GT) technology is crucial for introducing foreign genes from wild plants into legumes. Despite the low reliability of transgenic germplasms for reproduction, transgenic approaches enhance crop vigor and tolerance to abiotic and biotic challenges, improving productivity and quality (Somers et al. 2003). These tools aid in understanding transcriptional activation mechanisms linked to stress resilience. Agrobacterium mediated transformation is the most useful and powerful technique applied in legumes for genetic transformation followed by microprojectile bombardment (MP), direct gene transfer to protoplast (DGTP), and electroporation (EP) (Ali et al. 2022). Despite complexities in genetic pathways, genetically modified legumes show increased resilience to salinity, drought, and metals (Atkinson and Urwin 2012; Sita and Kumar 2020). Overexpression of the *MuWRKY3* gene in groundnut (*Arachis hypogaea*) improved drought tolerance in transgenic plants compared to wild-type plants. The nuclear-localized TF *MuWRKY3* modulated stress-responsive gene expression and ROS scavenging enzyme activity, which resulted in increased drought tolerance, suggesting that *MuWRKY3* may serve as a CG for stress resistance improvement in legumes and other plants (Kiranmai et al. 2018). In soybeans, overexpression of the *GmSAMT1* gene provided resistance against the soybean cyst nematode, *Heterodera glycines Ichinohe* (Lin et al. 2016). Moreover, soybean plants transformed with Arabidopsis DREB genes (*AtDREB1A*, *AtDREB2CA)*, and *AtAREB1* displayed promising agronomic and physiological responses to drought in comparison to the wild type (Fuganti-Pagliarini et al. 2017).

3.5 New Plant Breeding Techniques (NPBTs)

Advancements in molecular biology, genetic engineering, and omics technologies, combined with insights from the literature, have facilitated the successful employment of several modern breeding techniques, such as MAS, gene pyramiding, and speed breeding (Fig. 2), in crops such as rice, wheat, and maize. However, legumes are yet to fully leverage these molecular breeding methods, primarily due to the limited availability of data beyond diversity and QTL mapping studies (Salgotra and Stewart 2022). The ongoing and future whole-genome sequencing projects are expected to revolutionize crop improvement efforts. With the availability of genomic data, the precise knowledge of gene expression patterns, stress regulation mechanisms, and the identification of key stress response components, a suite of new breeding techniques (NBTs) have been developed. NBTs improve the efficiency and precision of plant breeding to improve desirable traits, which otherwise could be tedious and time-consuming via classical breeding alone (Table 5). These specific changes in the plant genome

can vary in scale, from altering a single base to changing multiple genes. NPBTs include genome editing techniques such as oligonucleotide directed mutagenesis (ODM) and sequence specific nuclease (SSN), transformation methods such as cisgenesis and intragenesis, grafting, and agro-infiltration, manipulation of the epigenetic landscape via RNA-dependent DNA methylation (RdDM) and breeding techniques such as precision breeding and reverse breeding (Enfissi et al. 2021). Products from NBTs range from genetically modified (GM) crops to non-GMOs. NBTs can induce small targeted modifications in the plant genome and modify gene expression without changing the genetic code through epigenetics.

Table 5 Conventional and new breeding techniques

Conventional Breeding	**New Breeding Techniques (NBTs)**
Mass selection, Pure line selection, Hybridization, Backcrossing, Mutation breeding, Polyploidy induction	ODM, SSN, cisgenesis and intragenesis, grafting, agro-infiltration, manipulation of the epigenetic landscape RdDM and reverse breeding
Dominant genes are typically chosen, Recessive allele selection is a more tedious process	The advent of Genomic resources and HTP lead to the identification of gene locations, thus, recessive alleles can be discovered in a relatively quick way
It is carried out physically by local breeders using basic tools, making it less accurate	Advanced technologies like MAS, GS, HTP are used
Lengthy breeding cycles, requiring multiple generations for trait introgression.	Rapid development of stress-tolerant varieties due to targeted gene editing and precise trait modification.
Limited precision in trait selection, often resulting in linkage drag and unintended traits.	High precision in trait modification, allowing for targeted gene editing.
Relies on natural genetic variation present within breeding populations.	Allows for the introduction of novel genetic variation from diverse sources, including unrelated species.
Limited flexibility in modifying complex traits or introducing multiple gene combinations simultaneously.	Offers greater flexibility in modifying complex traits and stacking multiple beneficial genes in a single transformation event.
Relatively high costs associated with maintaining large breeding populations and conducting field trials.	Initial investment in technology and infrastructure may be required, but long-term costs can be lower due to reduced breeding cycles and field trials.

Genome editing is a revolutionary and accurate genetic engineering technology that modifies specific target genes, playing an important role in the characterization of gene function and crop improvement (Taranto et al. 2018). Somaclonal variations, tissue culture, MAB, mutagenesis, wide hybridization, DH, and GT are the main tools of genome editing (Popelka et al. 2005; Aasim et al. 2011). Three main types of genome editing techniques viz. zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the CRISPR/Cas system, allow targeted and site-specific gene editing without inserting foreign DNA (Zhu et al. 2017). Numerous CGs associated with both biotic and abiotic stress have been explored using these GE techniques. In soybean, many genes associated with heat (*GmHsp90A2*), salt (*GmMYB118*, *GmAITRs*, *GmNHX5*, *GmNAC06*, *NTR1*, *PDH5*), drought (*GmMYB118*, GmNAC08, *NTR1*), and resistance to the soybean mosaic virus (*GmF3H1*, *GmF3H2*) have been characterized using the CRISPR-cas technique (Xu et al., 2013; Sun et al. 2021; Wang et al. 2021; Li et al. 2020; Yang et al. 2020; Huang et al. 2019; Du et al. 2018; Manjulatha et al. 2013; Xue and Zhang 2007). Furthermore, Double-stranded RNA-binding2 genes viz. GmDrb2a (Glyma.12g075700) and GmDrb2b (Glyma.11g145900) genes crucial for drought and salt stress resistance in

soybean were modified using CRISPR/Cas9 and TALENs genome editing methodologies (Curtin et al. 2017). In chickpea protoplasts, the CRISPR/Cas9 technique was used to edit 4-coumarate ligase (*4CL*) and Reveille 7 (*RVE7*), the drought stress tolerance associated genes (Badhan et al. 2021). Furthermore, genome editing also holds promise for enhancing shelf life and nutritional quality in leguminous microgreens like chickpeas (Mahto et al. 2022).

4 Future Perspective

The urgent need for robust legume cultivars with stable yields in challenging environments is clear. Legume genomes offer potential for identifying stress-tolerance genes, aiding transgenic or breeding methods. Recent studies identified markers and genes for improved growth and yield in different stress conditions, yet understanding stress adaptation in legumes is incomplete. Further exploration into stress-responsive genes, molecular components, and gene expression dynamics at different stages is vital. Bioinformatics aids omics data accessibility, urging comprehensive databases tailored for legumes. Incorporating a compendium of contemporary breeding methodologies, such as multi-omics platforms, genomic selection (GS), and expedited cultivation techniques coupled with high-throughput phenotyping, holds substantial promise for advancing our existing understanding of stress response mechanisms in leguminous plants. Leveraging genetic diversity inherent in stress tolerance traits derived from wild progenitors and traditional landraces is imperative for optimization in breeding initiatives. Future efforts merging genomics, transcriptomics, proteomics, metabolomics, agronomics, and CRISPR-Cas9 are crucial for resilient legumes thriving in diverse stress conditions. However, to date, very limited research has been conducted on the biotechnological perspective in leguminous crops including microgreens. Utilization of several omics' technology, genetic engineering, gene editing approaches along with advanced plant breeding technologies could be beneficial for enhancing stress tolerance and nutrition, ensuring they remain a viable and health-promoting food source under different environmental conditions. Furthermore, the already identified and newly discovered candidate genes involved in abiotic and biotic stress responses may also help improve microgreen traits.

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Management of Disease Losses in Leguminous Microgreens

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Abbreviations

BGs Baby Greens

CEA Controlled environment agriculture

DAS-ELISA Double Antibody Sandwich Enzyme Linked Immunosorbent Assay

DNA Deoxyribonucleic acid

EPPO European Plant Protection Organization

ISF International Seed Federation

ISTA International Seed Testing Association

ITS Internal Transcribed Spacer

JKF Jute Kenaf Fiber

LAMP Loop-mediated amplification

LED Light Emitting Diode

LMGs Leguminous MGs

MAS Marker Assisted Selection

MFP Macro Perforated Packaging

MGs Microgreens

mM Milli Molar

MNV Murine norovirus

MPN Most probable number

NGS Next Generation Sequencing

PCR Polymerase Chain Reaction

PET Polyethylene Terephthalate

PGPR Plant Growth Promoting Rhizobacteria

PPM Parts Per Million

PPP Polyethylene Packaging

RPB1 RNA polymerase B (I)

RPB2 RNA polymerase B (II)

RTF Recycled Textile Fiber

SEM

Scanning Electron Microscope

SFCM Soil Free Cultivation Matrices

STG Sure To Grow

USFDA United States food and Drug administration

UV Ultra Violet

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1 Introduction

Microgreens (MGs), characterized as young cotyledon greens, are typically harvested after 1–2 weeks of seedling emergence. Serving as a source of diverse dietary nutrients, MGs offer essential & nonessential elements to consumers. The nutrient composition varies based on the microgreen type, cultivar, and growth stage. Recent trends indicate a significant rise in microgreen consumption due to their tender texture, distinctive flavour, and nutritional content, including vitamins, antioxidants, and fibers & minerals, in contrast to mature leafy vegetables (Gupta et al. 2023). Classified as “functional food,” MGs possess health promoting characteristics besides the usual benefits of nutrition (Kou et al. 2013; Tallei et al. 2024). However, the microbial risk linked with MGs has not been thoroughly investigated. Leguminous MGs (LMGs) are protein rich food and have potential to cure all levels of protein deficiency in developing regions across the globe where animal proteins are unavailable/unaffordable (Xiao et al. 2012; Kou et al. 2014).

LMGs represent a raw salad component similar to sprouted seeds, albeit with distinctions in their production process compared to sprouts. The germination of LMGs spans approximately 72 hours in a soil, soil substitute or hydroponic nutrients. Following germination, LMGs undergo a growth period lasting around 2–3 weeks, culminating in the emergence of the initial set of true

leaves. Within controlled environment agriculture (CEA) systems, LMGs stand out as a lucrative and widely cultivated crop, constituting a significant segment of the CEA industry. It is noteworthy that the conditions in which LMGs are cultivated can impact the plant's susceptibility to bacterial uptake, potentially involving human pathogens that contribute to foodborne illnesses associated with produce. Up to the present time, LMGs have not been directly linked to any noticeable outbreaks. However, seven products have been recalled in the United States and Canada since 2016 due to *Salmonella spp*. and *Listeria monocytogenes* infestation. It is crucial to recognize that human and plant pathogens often exploit the plant root system, rendering post-harvest washing ineffective. Many variables, such as the kind of pathogen, growing medium, moisture content close to the rhizosphere, routes of contamination (for instance, irrigation or seeds etc.), and plant variety, can influence pathogen internalization. Additionally, alternative pathways such as stomata, wounds, and cuts have also been identified as potential routes for pathogen entry (Deng et al. 2021; Deng and Gibson 2023a, b).

LMGs find use in fresh food markets and home cooking, necessitating careful postharvest handling techniques for both industrial and domestic storage. Rigorous safety guidelines and compliances are essential for retaining the nutritional value and improving the shelf life of LMGs while preventing contamination (Galieni et al. 2020). Effective control measures against microbial contamination throughout production, postharvest handling, and storage are essential for ensuring food safety. Although no outbreaks have been reported, prioritizing microgreen food safety remains crucial. Inadequate handling may result in nutrient loss (Turner et al. 2020), underscoring the importance of proper care and safety measures for LMGs.

In fact, multiple routes of infections have been recorded; perhaps infected seeds are the most dominant contributor and frequent source of infection. Thereafter, another potential cause of contamination during preharvest growth is irrigation water. Seedling emergence offers an optimal habitat for microbial growth (foodborne outbreak might occur from only as 4 most probable number (MPN) of *Salmonella*/kg of seed). (Riggio et al. 2019). Unlike sprouts, thus far, no foodborne outbreaks linked to LMGs have been reported. The microbiological threat associated with LMGs hasn't been thoroughly investigated, yet. The healthy life of LMGs depends on factors such as the initial microbial load, relative humidity; exposures and susceptibility to postharvest pathogenic invasion are critical considerations. Foodborne pathogens pose significant health risks, with worker hygiene, contaminated irrigation water, production site sanitation, and equipment cleanliness being major factors affecting microgreen safety. As per reports, contaminated seeds represent a primary origin of contamination and possible infections (Riggio et al. 2019). Consequently, thorough decontamination procedures are essential to eliminate the risk of microbial infestation among consumers (Işık et al. 2020). This chapter provides an overview of the latest developments in micro green disease management methods during the production phase and postharvest.

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2 Potential Sources Affecting Diseases Losses in LMGs

Various types of diseases pose risks to LMGs, including microbes. As perishable items, fresh products are susceptible to contamination at every stage from farm to fork. LMGs, being immature at harvest, are particularly vulnerable to pathogen internalization and colonization due

to factors such as production and postharvest conditions (Fig. 1) (Turner et al. 2020).

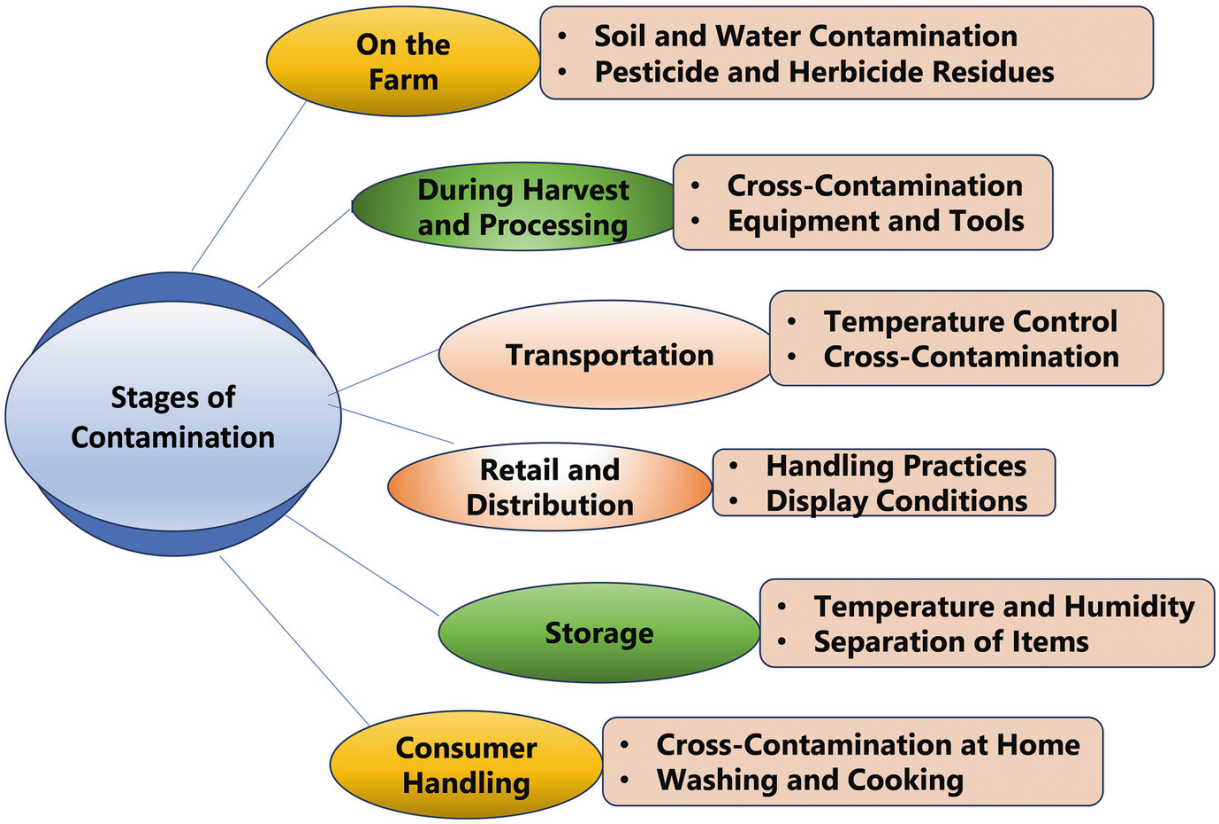


Fig. 1 Major contamination sources and stages of LMGs (production to consumption)

Contamination in LMGs originates from two primary sources: production factors and postharvest conditions. During the production phase, major contamination sources include seeds (including seed coat nature and seed sterilization), irrigation water, soil amendments like animal manure and growing media. Apart from many, microbial infestations are the predominant cause of contamination in LMGs. Tissue damage, inappropriate harvest, and farmworker hygiene are crucial aspects that contribute to microgreen contamination (Riggio et al. 2019). Microbial counts on LMGs vary, emphasizing the need for effective decontamination strategies. The delicate watery tissues with soft texture of LMGs create favorable conditions for

microbial growth (Chandra et al. 2012). These conditions must be considered when devising decontamination strategies to assure the food safety (Kou et al. 2013). LMGs are extremely susceptible to microbial invasion during production, harvesting, and marketing (Xiao et al. 2015).

Although animal intervention and soil amelioration are underestimated minor factors due to short time and indoor production, contaminated production locations and equipment pose significant risks to microgreen safety (Riggio et al. 2019; Işık et al. 2020). Investigation into microbiological contamination and *Salmonella* survival on Swiss chard MGs demonstrated that the host affects the functional diversity of pathogens in the rhizosphere (Reed et al. 2018).

LMGs, distinguished by their perishable nature, exhibit a considerably shorter shelf life in contrast to leafy vegetables (Kou et al. 2014). Despite being kept under refrigerated conditions, LMGs have a relatively brief shelf life, often produced in small quantities. Understanding the relationship between spoilage and pathogens is crucial in addressing these challenges.

The delicate nature of LMGs makes tissue damage during harvest and postharvest processes a critical factor contributing to contamination in these ready-to-eat greens. Furthermore, the use of contaminated packaging materials can also lead to infestations (Riggio et al. 2019). Microbial infection is facilitated by enzymatic digestion of cuticle, which contribute significantly to LMGs contamination. While the cuticle provides a certain level of protection against contamination, advanced research is needed to establish a robust correlation between the composition of the cuticle and microbial infection (Gao et al. 2016). Ongoing research is essential to delve deeper into these factors and enhance our understanding of LMGs safety measures.

2.1 Effect of LMGs Production Systems on Incidence of Diseases

Xiao et al. (2015) demonstrated the proliferation and survival of *Escherichia coli* in hydroponic and soil-substituted radish MGs, noting a higher infestation rate in the hydroponic system in contrast to the soil-substitute. Interestingly, fiber mats were found to alleviate multiplication of pathogens in contrast peat alone and peat based mixes indicating the advantages of recycled fiber mats over peat (Di Gioia et al. 2017). However, efficiency of recycled mats is dependent upon the conditions of their recycling, and there is no inheritability of pathogenic survival.

Reed et al. (2018) recorded the growth pattern of different media to assess serovar-dependent *Salmonella* production on MGs. Hydroponic pads showed the maximum population of *Salmonella* compared to other media when contaminated water was applied. The study also observed the proliferation of a seed-borne microbe *L. monocytogenes*, in both hydroponic and soil-grown MGs. Moreover, decaying of the seed coat owing to pathogen growth was noted in hydroponic and soil cultures, with a similar proliferation pattern observed in the edible portion of the MGs. Maximum contamination was found on cotyledons, seed coats and roots (Wang et al. 2015).

As per reports, the internalization ability of human norovirus surrogate and murine norovirus (MNV) was investigated in kale and mustard MGs, revealing a reviving pattern of virus survival rates even MGs were irrigated with disinfected water (Wang and Kniel 2016). Significantly high viral load occurred in every edible part of both MGs, with MNV translocating via roots even after 12 days of contaminated water application. In the same context, bacterial spatial contamination patterns on seeds were monitored, showing initial contamination of both roots and

aerial parts, leading to whole plant deterioration at later stages.

Furthermore, the efficacy of bactericidal LED (blue and UV ranges) light for food safety was highlighted, for treating water that is utilized in hydroponically grown MGs (McKenzie et al. 2014; Kim et al. 2016). Despite efforts to sanitize plant tissues, still no foolproof approach is available to eradicate pathogens after contamination due to the sensitivity of LMGs. Sanitization processes face limitations, and food surface morphology plays a crucial role. In another context, the survival patterns of the O157:H7 strains of *E. coli* were found in various MGs during refrigeration, with minute changes noted in the yield rate after a week of inoculation. Higher proliferation rates were observed on true leaves compared to mature leaves, and scanning electron microscope (SEM) analysis revealed little longer stomata in MGs in contrast to mature leaves indicating infestation (Park et al. 2013).

2.2 Safety Status of LMGs Production

LMGs share several properties with baby greens (BGs) and sprouts (Turner et al. 2020). These are continuously gaining popularity as functional foods. Therefore, appropriate postharvest handling techniques are essential for storage (domestic and industrial), along with strict safety protocols to preserve valuable nutrients and prolong the shelf life of LMGs (Galieni et al. 2020).

LMGs are produced in various environmental conditions (open air, indoor and controlled protected environment) and growing systems (soil & soilless). Controlled protected environments, often free of contamination, help ensure the quality of LMGs. Production systems are tailored based on the scale and quantity required, with many growers producing LMGs for commercial or household use. Mixed

microgreen production (interfamilial or intraleguminous) is also a common practice among growers (Mir et al. 2017).

Accomplishment of production relies on harvesting at the correct stage, as improper harvesting can alter quality and tenderness. Harvest timing varies based on the crop and production system. Growers must choose varieties with rapid and uniform growth characteristics to enable simultaneous harvesting. Recurrent harvesting may introduce contamination risks due to bad laboratory practices, equipment's, and anthropogenic activities into the production facility. Sterilizing harvesting equipment is crucial to prevent contamination and potential foodborne outbreaks. LMGs are commonly produced in containers, garden beds, or on windowsills, using various growing media such as standard soil, bark, peat moss, perlite, vermiculite, coco coir, rock wool, and renewable materials like cotton, jute and cellulose pulp. Beneficial microorganisms may be added to the growing media to enhance vegetative growth and control of infectious agents (McGehee et al. 2019).

As standard practice, seeds are sown in rows or by using the broadcast method, with some growers utilizing a nutrient-based film. Thick seeding is common for maximizing yield, but excessive density can lead to elongated, crowded, and etiolated growth patterns, increasing susceptibility to diseases. Fertilizers, especially organic amendments, can boost yield of MGs, but certain organic fertilizers may increase susceptibility to foodborne pathogens, challenging food safety principles. Therefore, safety measures and adherence to essential protocols and standard operating procedures during the production phase are crucial to mitigate possible contamination risks in LMGs.

2.3 LMGs Susceptibility to Diseases at Harvest, Packaging, Marketing

LMGs are typically harvested when they reach the requisite height, and the emergence of the first cotyledon and true leaves. As per reports, the timing of harvest varies in accordance with variety, and it is generally recommended to harvest LMGs within 10–20 days after sowing (Riggio et al. 2019). Due to their highly perishable nature, fresh LMGs must undergo thorough washing and quick cooling using sustainable methods designed to ensure protection against microbes. LMGs are commonly packed in polyethylene packaging (PPP) bags after harvesting and refrigerated to maintain freshness before being transported to markets (Mir et al. 2017). In another context, container-based production is adaptable for LMGs at commercial as well as micro-scale urban scale, allowing for rapid commercialization. This approach is advantageous, especially for small container-based production, as the container can be directly routed to markets, and consumers can harvest the LMGs themselves, bypassing the need for growers to handle the produce and reducing the risk of contamination (Kyriacou et al. 2016).

The storage of LMGs under specific light conditions (based on the growth stage and nature of the LMGs) has been reported to increase phytochemical contents and suppress potential microbial growth. The type of light used plays a crucial role in microbial suppression, with certain UV radiations having potential benefits in reducing microbial load (Martínez-Zamora et al. 2021). While certain light conditions (light quality and exposure time) offer aid, as well as possibly lead to detrimental effects. Therefore, cautious consideration is essential at market outlets to preserve the general eating quality and safety of LMGs. This aspect deserves thorough investigation for LMGs to ensure effective sterility during the production phase. Further research is needed to explore the impact of light conditions on sterilization and safety of LMGs during the postharvest phase.

3 Approaches for Disease Management in LMGs

3.1 Production-Based Approaches

3.1.1 Detection of Seedborn Pathogen and Management Approaches

The seed serves as the fundamental requirement for microgreen production, and ensuring sterility of seed is crucial due to the potential proliferation of pathogens, particularly in hydroponic culture techniques (Reed et al. 2018; Wright and Holden 2018). As contaminated seeds are primary source of pathogens and pose a major challenge in the production. Once internalized during germination, it becomes a challenge to eliminate them. For instance, the transmissions of murine norovirus have been reported in kale and mustard MGs under hydroponic system, emphasizing the importance of seed sterilization for LMGs (Wang and Kniel 2016). Different potential pathogens and Major diseases associated with LMGs at various stages of growth and processing are listed (Fig. 2 and Table 1).

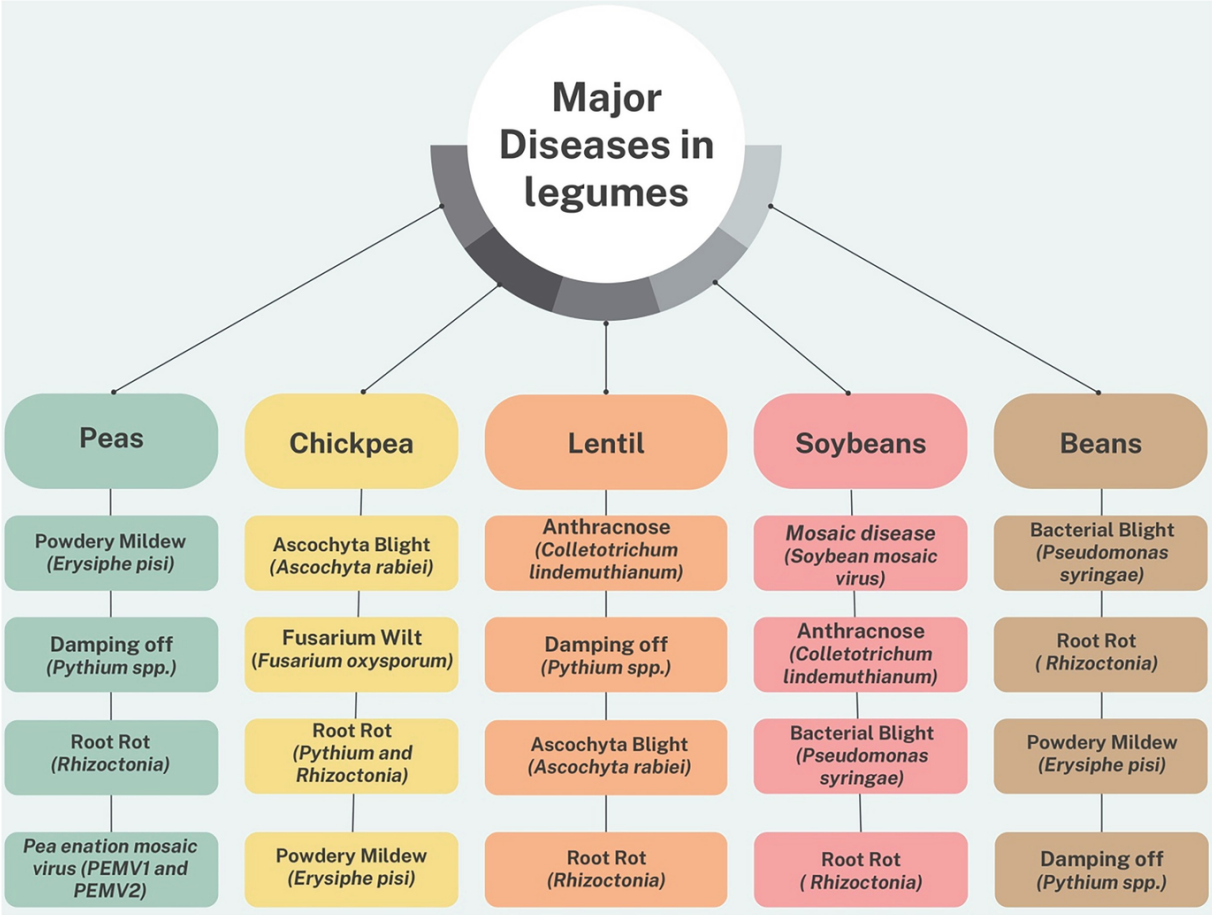


Fig. 2 Major diseases and pathogens of legumes may target LMGs (production to consumption)

Table 1 Major LMGs and their pathogens causing losses

Major LMGs	Fungus & moulds	Bacterial	Viral	Insect pest
Common bean (*Phaseolus vulgaris* L.) Cowpea (*Vigna unguiculata* L.) Chickpea (*Cicer arietinum* L.)	*Alternaria alternata* *Aspergillus flavus* *Aspergillus niger* *Aspergillus parasiticus* *Macrophomina phaseolina* *Aspergillus terreus* *Aphanomyces euteiches*	*Xanthomonas sp.* *Pseudomonas syringae* *Pseudomonas savastanoi* *Pseudomonas viridiflava* *Curtobacterium flaccumfacies*	*Alfalfa mosaic virus* *Cucumber mosaic virus* *Lucerne transient streak virus* *Lucerne australian*	Aphids (greenfly and blackfly) Whiteflies (*Trialeurodes vaporariorum*) Fungus gnats (*Bradysia coprophila*)

Major LMGs	Fungus & moulds	Bacterial	Viral	Insect pest
Faba-bean (*Vicia faba* L.) Groundnut (*Arachis hypogaea* L.) Lentil (*Lens culinaris* L.) Peas (*Pisum sativum* L.) Alfalfa (*Medicago sativa* L.) Clovers (*Trifolium* sp.) Annual medics (*Medicago* sp.)	*Sclerotinia sclerotiorum* *Fusarium sp.* *Mucor sp.* *Penicillium sp.* *Rhizopus sp.* *Trichoderma sp* *Epicocum sp.* *Botrytis* sp. *Colletotrichum* sp. *Ascochyta* sp. *Rhizoctonia solani* *Diaporthe/Phomopsis* complex		*latent virus* *Bean yellow mosaic virus* *Clover yellow vein virus* *Pea streak virus* *Bean common virus* *Bean common necrosis virus* *Tobacco Ringspot Virus* *Cowpea chlorotic virus* *Seedborne blackeye cowpea mosaic strain* *Cowpea aphid-borne mosaic virus* *Cowpea mosaic virus* *Cowpea mottle*	

Major LMGs	Fungus & moulds	Bacterial	Viral	Insect pest
			virus *Southern bean mosaic virus* *Pea seedborne virus* *Pea seedborne mosaic virus* *Pea enantion mosaic virus-1* *Tomato mottle mosaic virus* *Broad bean stain virus* *Pea early-browning virus* *Indian peanut clump virus* *Peanut clump virus* *Peanut mottle virus* *Tobacco streak virus*	

Major LMGs	Fungus & moulds	Bacterial	Viral	Insect pest
			Red clover mosaic virus *Soybean mosaic virus* *Tobacco ringspot virus*	

Various seed sterilization techniques are employed globally. In fact, the efficiency of seed sterilization rests on the variety and nature of the seeds. A standard chemical disinfectant method reported by the USFDA (United States Food and Drug Administration) involves soaking seeds in 20,000 ppm calcium hypochlorite (10–15 minutes). Viruses and bacteria may hide in the crevices on the surface of legumes, having irregular and rough surfaces in contrast to other vegetables (Riggio et al. 2019).

Besides sodium hypochlorite dipping, methods like heat treatment and application of high-pressure can also be utilized. High-pressure treatment has been identified as an effective physical method for seed sterilization with minimal impact on seed germination (Ding et al. 2013). A thorough investigation is necessary to evaluate the effectiveness of various sterilization techniques for LMGs and ensure their safety during production.

Visual examination and the use of a stereoscopic microscope prove valuable in observing the multiplication of fungi and other contrasting pathogens. An incubation method, a common diagnostic approach, is useful for high incidence pathogens. In this approach, surface sterilization is often included to eliminate surface pathogens. It is crucial to note that endophytes within seeds present the major challenge to seed health. Further, seeds are incubated on drenched filter paper, or on agar media, in

Petri dishes with test durations ranging from 2–10 days at temperature appropriate to target pathogen.

Fungal pathogens may develop into colonies below the seed coat, endosperm, or embryos, when surface sterilization is performed. Selective media, enrichment methods, have been developed to facilitate the isolation of seedborne pathogens. The final step involves microscopic visualization of the colonies and characterization of structures that are species-specific. In another context, incubation is also employed on the basis of soaking method. In this method, seeds are soaked in a suitable buffer followed by maceration for 2–18 hours at optimum temperature range. Bacteria released in this process are then plated on agar media (semi-selective), for the selection and isolation of bacterial pathogens residing inside the seed. Following the detection of putative pathogenic isolates, pathogenicity tests will be performed. Serological methods, employing polyclonal or monoclonal antibodies, offer a viable alternative for detecting seedborne infections. DAS-ELISA (double antibody sandwich enzyme linked immunosorbent assay) is a commonly used technique in this field, providing the merit of being relevant to diseases that are both necrotrophic and biotrophic. It is widely used to identify viruses that get transmitted via seeds. (Dell'Olmo et al. 2023).

Polymerase chain reaction (PCR) based molecular diagnosis methods have proved to be the most popular in the identification of seedborne pathogen due to prompt detection, specificity, sensitivity, and also simplicity of interpretation. PCR based detection methods require some initial steps in which DNA is extracted and purified. Some scientific lab still depends on conventional methodologies involving the establishment of fungal monosporic or monohyphal culture, which is followed by lyophilization and extraction using a readymade kit having chemical components. This Primers based detection method depends

on the ITS (Internal Transcribed Spacer), in this, large (nrLSU-26S or 28S), and small ribosomal subunits (nrSSU-18S) are used. ITS, with its considerable variability, is particularly suitable for species identification and combined with protein-coding genes for more precise molecular characterization. In this method the regions that are utilized frequently include the RNA polymerase's largest (RPB1) and second-largest (RPB2) subunits, β-tubulin (tub2/BenA), and translation elongation factor 1-α (tef1). Advanced molecular techniques include quantitative PCR, multiplex PCR, nested PCR, and also PCR based loop-mediated amplification (LAMP). Where in case of RNA Seq based NGS (Next Generation Sequencing), specifically offers rapid identification of fungal plant pathogens diseases. Serological methods are sufficient for routine testing in the diagnosis of pathogenic viruses, and molecular-based detection operates well for strain differentiation when high sensitivity is needed (Hariharan and Prasannath 2021).

In recent years, the market for Legume-Mediated Grains (LMGs) has been threatened by the spread of seedborne pathogens. Field inspection and diagnostic analyses serve as a continuous safeguard against mass devastation. Robust seed health testing methods are essential to support awareness about risks and phytosanitary emergencies. Seed diagnosis plays a vital role in managing disease losses against LMGs, contributing to plant health, development, and harvesting. Seeds that are pathogen free or have infection values lower than permissible limits may be considered healthy and suitable for planting. Despite the challenges posed by resistance genetics, pathogen variability, and a restricted gene pool, the development of resistant cultivars is one of the main schemes for reducing the effects of seedborne infections.

The microbial population's complexity and strain variability restrict the resistance genes being introduced

into different varieties of legumes. Advancements in breeding, facilitated by MAS (marker assisted selection) and an improved understanding of the transcriptome, reveal that resistance is linked to the upregulation of salicylic acid and the downregulation of photosynthesis.

The choice of treatment to counteract seedborne diseases based on where in the seeds the pathogen is located. Surface contaminants are controlled by contact fungicides, while translaminar or cytotropic fungicides are effectively used to eliminate minor infections in the seed layers on the exterior. For infections involving inner seed layers (embryos), only systemic compounds can prevent the disease. Commonly used compounds for seed treatment belong to various chemical groups such as triazole, dicarboximide, carboxin, acylalaninate, benzimidazole, and strobilurins.

Natural extracts such as thyme and garlic essential oils have shown promise in inhibiting plant pathogen growth. Treatments with Trichoderma spp. or Plant-Growth-Promoting Rhizobacteria (PGPR) enhance germination rates and reduce the incidence of seedborne diseases. Biological formulations against various pathogens, including *Bacillus* strains, *Gliocladium catenulatum*, and *B. subtilis*, demonstrate effective disease management in legume crops. These findings provide promising ideas concerning environmentally sustainable handling of disease losses in LMGs (Dell'Olmo et al. 2023).

3.1.2 Sterilization of Growth Media

Growing media can be a direct source of pathogens for LMGs, during production. Investigating the effects of bottom and spray-irrigated MGs (radish and lettuce) grown on perlite and peat moss (inoculated with Escherichia coli 0157:H7), it was shown that MGs, independently of the growing medium, had considerably greater levels of *E. coli*

and *E. coli* 0157:H7. This suggests the need for strict regulations to mitigate *E. coli* based microbial risks when cultivating LMGs on growing media, especially perlite. It emphasizes the importance of addressing seed decontamination, including postharvest cleaning procedures and irrigation water quality to ensure the security of LMGs. (Işık et al. 2020).

In another study, the impact of different growing media, including sure-to-grow (STG), jute-kenaf-fiber (JKF), peat, and also recycled textile fiber (RTF), on rapini or broccoli MGs was analyzed. At harvest, the aerobic microbial population was unaffected by substrates; however, mold and yeast counts were higher in JKF and peat-grown MGs than in RTF and STG. *E. coli* and Enterobacteriaceae were significantly higher in rapini MGs grown in peat, and *E. coli* was not detected in MGs grown in peat compared to other media. This underscores the importance of thoroughly sterilizing growing media before sowing LMGs. Additionally, more studies are required to determine how growing media sterilization affects microbial populations throughout and after harvest; this is a topic that has not yet received much attention but has to be done in the future (Di Gioia et al. 2017).

3.1.3 Irrigation Water

LMGs, although often cultivated in controlled environments like high tunnels and greenhouses, remain susceptible to potential contamination from various sources. Irrigation water, particularly in hydroponic microgreen production is a major concern. Contaminated irrigation water can introduce viruses such as norovirus to LMGs, posing a threat to food safety (Riggio et al. 2019). Different types of irrigation water, including sewage and wastewater, are utilized in production, with sewage water being recognized as a source of pollutants and microbes (Naz et al. 2021). In

controlled environments like greenhouses, LMGs may be irrigated with water from sources such as groundwater, collected rainwater, municipal sources, or gray water. The quality of water varies based on its source, and the selection of an appropriate water source is crucial to minimize the risk of foodborne pathogens. Municipal water, commonly used for irrigation, has been identified as a carrier of certain foodborne pathogens, emphasizing the need for careful consideration in choosing the water source for microgreen irrigation (Uyttendaele et al. 2015).

3.1.4 Use of Biofungicides for Sterilization of LMGs

In addition to bacterial infestation, fungi can also pose contamination challenges during production in LMGs. Pythium species, causing damping-off and root rot, are among the leading causes of contamination in MGs. These biofungicides *B. subtilis* GB03, *B. amyloliquefaciens* D747, *Trichoderma harzianum* KRL-AG2, and *Trichoderma virens* G-41 effectively reduced root rot and damping-off in radish, kale, arugula, and mustard MGs caused by *Pythium dissotocum and Pythium aphanidermatum*. However, the application of biofungicides resulted in reduced plant biomass, necessitating an extended production period for growers to achieve the targeted yield of MGs. Further research in this area is needed to recognize the role of biofungicides in both the production as well as decontamination of LMGs (McGehee et al. 2019).

3.1.5 Use of Soil-Free Cultivation Matrices (SFCM)

Furthermore, the SFCM has been demonstrated to influence pathogen persistence over time. The effect of SFCM on pathogen persistence is found significant during microgreen growth; however detailed study is lacking. In

conclusion, current research reaffirms that the plant variety used can affect the survival of foodborne pathogens on MGs. Relevant data on the pathogens' fate in commonly grown microgreen varieties grown on biostrate and peat SFCM is generated by this study. As more data about the relationship between SFCM types, microgreen varieties and pathogen persistence become available, growers are able to evaluate the food safety hazards in microgreen cultivation processes which will have implications for the industry (Misra and Gibson 2020).

3.1.6 Seed Density and Seedling Pathogens

High seed and seedling density, preferable environmental conditions, and phases of development seedling, create an environment conducive to the proliferation of a diverse range of pathogens on MGs (Verlinden 2020). Pathogens like *Pythium spp.* can lead to seed, root, and seedling rot during the short production phase, and the edible nature of LMGs poses challenges for the use of conventional fungicides. In response to these challenges, alternative control measures have been explored, with biocontrol agents such as *Trichoderma* species showing promise for disease management on LMGs. Research indicates that higher rates of *Trichoderma* not only contribute to disease control but also enhance yield, possibly because of improved seedling survival rates (Pill et al. 2011).

Another approach involves using bacteria as a seed treatment to promote the growth of LMGs. The application of endophytic bacteria as a seed treatment may increase dry and fresh biomass of LMGs, potentially attributed to reduced seedling mortality and suppressed microbial infection as demonstrated in other MGs. While this effect could be beneficial for reducing microbial infection and extending the shelf life of LMGs, more research is needed in this area (Briatia et al. 2017; Verlinden 2020). Washing

and drying treatments for seed treatment may result in mechanical damages on microgreen leaves and hypocotyl regions, serving as potential entry points for microbes. Further exploration and research in seed treatment methods for LMGs are crucial for developing effective strategies to promote growth and control diseases during production.

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4 Non-production Based Techniques

4.1 Wash for LMGs Sterilization

Antimicrobial-based wash treatments, especially those with chlorinated water, have shown potential in minimizing microbial burdens on MGs, with a focus on populations of aerobic mesophilic bacteria. (Chandra et al. 2012). For instance, a significant reduction was noticed in the count of aerobic mesophilic bacteria on buckwheat MGs compared to the control with a 100 mg/L chlorinated wash treatment (Kou et al. 2013). However, the efficacy of wash treatments can vary depending on the nature of the produce. In the case of radish MGs, a chlorinated water wash with the concentration 100 mg/L of did not reduce the count of aerobic mesophilic bacteria efficiently (Xiao et al. 2014a, b, c). In case of LMGs water treatment doses need to be optimized for post-harvest sterilization.

The effectiveness of antimicrobial wash treatments is often influenced by the duration of application. For an example, the population of bacteria rebounded in water-washed buckwheat MGs only just after 21 days of storage. Similar trends were observed in mold and yeast on broccoli MGs. Several factors have been identified as contributors to microbial rebounding, including water-soaked lesions on the hypocotyls of MGs (Kou et al. 2013, 2015). Overcoming microbial rebounding is a significant challenge in achieving efficient sterilization of LMGs during the postharvest stage.

The residual contents and mechanical damages can limit the extension of shelf life for LMGs (Kyriacou et al. 2016). Additionally, the consideration of maximal residual concentrations of the selected agents used in wash treatments is an aspect that has been overlooked. Investigating this aspect is important to ensure the safety of LMGs by minimizing the risk of higher chemical uptake and potential health concerns.

4.2 Sterilization of LMGs by Calcium Salts

In a study on broccoli MGs, the treatment with calcium chloride remarkably decreased microbial infection; particularly microbes grow in oxygenated environments including *Staphylococcus aureus*, and *E. coli* in terms of aerobic mesophilic bacteria. A concentration of 10 mM L^{-1} calcium chloride was found to be the most efficient for broccoli MGs, contributing to enhanced shelf life and preserved quality by minimizing the incidence of aerobic mesophilic bacteria (grow between 20 and 45 °C) over a 21-day storage period (Kou et al. 2014). Another study reported that the application of 10 mM L^{-1} calcium chloride ($CaCl_2$) significantly inhibits the microbial infestation of aerobic mesophilic bacteria as well as total mold and yeast in broccoli LMGs. It's noteworthy that calcium was treated both during the production phase and postharvest storage. Calcium chloride when combined with calcium lactate, found to be more efficient in preserving the overall quality and extending storage life compared to their individual applications. Furthermore, the postharvest application of calcium lactate dip was recoded to considerably lessen the infection of microbes on harvested broccoli MGs. However, it is crucial to note that mechanical damage such as washing and drying may have negative effects if not handled properly, especially for delicate LMGs (Kou et al. 2015).

4.3 Sterilization of LMGs by Organic Acids and Ethanol

Dipping treatment methods are efficient to reduce the microbial load of LMGs. Chandra et al. (2012) demonstrates that, the application of a 0.5% citric acid combined with a 50% ethanol was found to be as effective as a sodium hypochlorite (NaClO)-based treatment in disinfecting and inhibit the proliferation of aerobic mesophilic and coliform bacteria on Chinese cabbage MGs stored for 9 days at 5 °C. Another MGs investigation on sunflower explored the effect of 0.5% citric acid, ethyl alcohol vapor (100%), and 0.5% ascorbic acid either alone or in combination on 16 days of storage at 10 °C. A scientific experiment performed by Dalal et al. (2020) based on ascorbic acid treatments, either alone or in combination with citric acid, findings that showed the greatest promise for increasing the shelf life of sunflower MGs were discovered. In the context of deceasing virus contamination, the application of peracetic acid was explored on arugula MGs. Treatment with 30 or 80 ppm peracetic acid for 3 minutes, resulting in a positive reduction of both Rotavirus and Tulane virus infestation. Fuzawa et al. (2021) explained that the effectiveness of peracetic acid-based sanitization treatments were found to depend on the location of the viruses in the MGs (Fuzawa et al. 2021). The above mentioned strategies are supposed to be effective for LMGs also.

4.4 Edible Coating for LMGs

Edible coatings have been recognized to limit diverse types of microbes on MGs, depending on the nature of the coating used. The study found that Aloevera gel coating, when applied as a preharvest foliar spray, lowered down the microbial number in roselle and radish MGs significantly as compared to the postharvest dipping of the

same coating. Additionally, the Aloe vera gel coating spray contributed to preserving the edible quality of MGs for 8 days under 5 °C storage conditions (Ghoora and Srividya 2020). Notably, the effect of pre-harvest application of edible coatings on postharvest microbial control is relatively minimal, and further research is needed. Future investigations should explore the efficacy of various coatings on different LMGs to enhance our understanding of their potential in improving postharvest safety and quality.

4.5 Packaging Material for LMGs

The storage conditions (carbon dioxide levels and oxygen concentration) play a crucial role in decay and microbial infestation of harvested LMGs. The extension of LMGs' storage life can be achieved through the use of suitable packaging. The potential of polypropylene packaging (PPP) bags in minimizing microbial infestation of MGs was investigated. Various microbial groups, such as mesophilic aerobes, enterobacteria, streptococci, listeria, lactic acid bacteria, pseudomonas, yeasts, staphylococci, and molds, demonstrated substantial alteration during PP packing. (Paradiso et al. 2018). Careful attention to both gaseous composition and storage temperature is essential to control microbial proliferation and ensure the sterility of LMGs before consumer use. It's important to note that sub-optimal storage temperatures may encourage microbial growth due to chilling injury (impair cell membranes, increase ion leakage, and enhance senescence), leading to the formation of water-soaked lesions, serve as a medium for microbial proliferation (Ali et al. 2019, 2021).

Recently, the impact of low-density polyethylene seal bags, macro-perforated packaging (MFP), and polyethylene terephthalate (PET) clamshells were studied on MGs. PET clamshells resulted in significantly lower microbial counts, extended shelf life, and better consumption quality during

storage at 5 °C for 8 days compared to the control roselle and radish MGs (Ghoora and Srividya 2020).

4.6 LMGs Sterilization by Ultra Violet Radiations

Ultraviolet (UV) radiation, including (UV-C & UV-B) has demonstrated to increase nutrient contents and microbial infestation on harvested MGs. The impact of UV-B & C, either alone or in combination was investigated on broccoli and radish sprouts. The epiphytic microbial load which was found notably lower by UV-C treatment in contrast to other treatments (Martínez-Zamora et al. 2021). In current, there is no research available, that specifically focusing on the application of UV radiation to ensure sterility in LMGs. Therefore, it is put forward to conduct comprehensive investigations into the impact of UV radiation throughout the production and post-harvest stages. Further research could provide valuable insights into the potential of UV radiation for sterilization of LMGs in the near future.

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5 Discussion

A key factor in limiting the spread of seedborne diseases in different environments is seed fitness, emphasizing the pivotal role of preventive diagnosis in managing losses. Given that the majority of pathogen-infected seeds exhibit no symptoms, and only a small percentage of these infections manifest as symptomatic plants, effective diagnostic methods are essential. Collaborative efforts by the International Seed Testing Association (ISTA), International Seed Federation (ISF) and European Plant Protection Organization (EPPO) have led to the establishment and validation of various protocols integral to seed certification processes (Dell'Olmo et al. 2023).

In summary, many studies have been done to develop a unified strategy for the efficient management of disease losses in MGs (Fig. 3 and Table 2). Although approaches to manage pre-germination, germination to post harvest microbial load may vary on the basis of crop, variety and pathogenic strain. In present scenario, LMGs are susceptible to microbial exposure at any stage that can compromise their safety and yield. To ensure sterilization of LMGs, it is crucial to implement appropriate protocols throughout production, harvest, and postharvest handling. Key underestimated sources of contamination in LMGs include wildlife feces, surrounding vegetation, soil amendments, worker health, as well as non-compliance of good laboratory practices. These factors pose potential food safety challenges for LMGs if not managed carefully. Various sterilization techniques, both during production and postharvest, have been employed for LMGs. However, there is a need for comprehensive and detailed research in crop/variety specific manner to fully reveal the mechanisms and prospective of different sterilizing agents in the context of LMGs. Additionally, exploring decontamination techniques that have proven effective for baby vegetables and sprouts in the case of LMGs would be beneficial. Continued research in this area will contribute to enhancing the safety of LMGs to achieve food security as one of sustainable development goal.

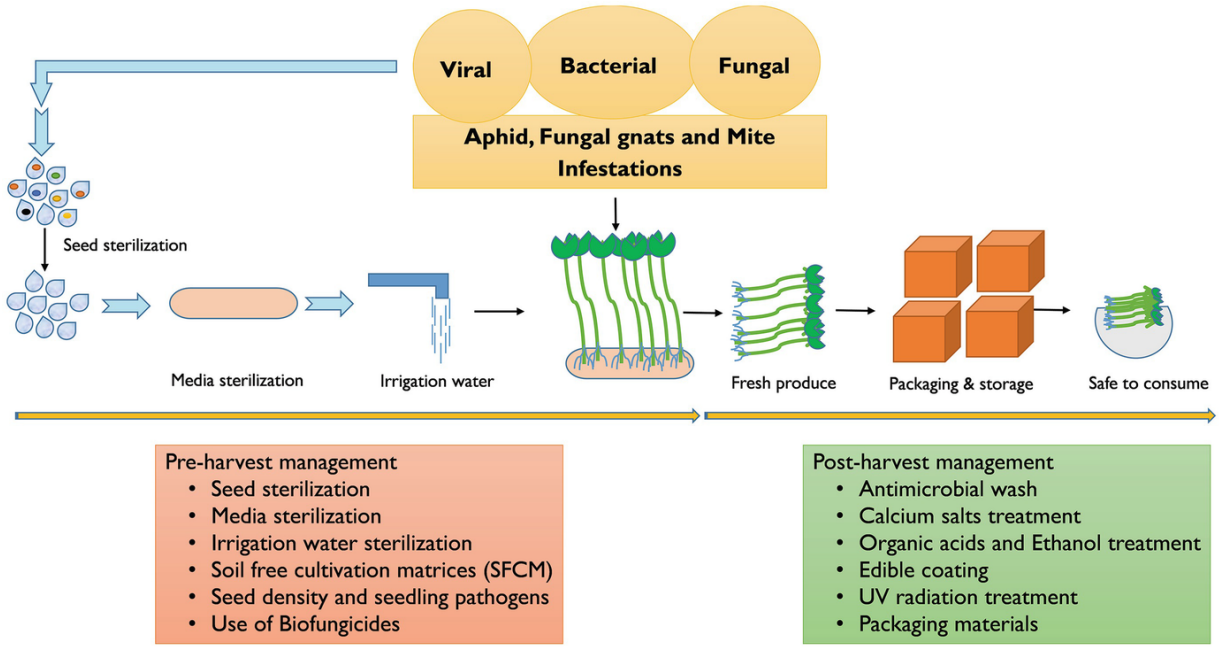


Fig. 3 Schematic representation of factors associated with diseases losses in LMGs and their efficient management from seed to table

Table 2 Good laboratory practices to manage disease losses in LMGs

S. No.	Pathogen	Proposed management to reduce losses due to disease
1.	Fungal & moulds	Keep microgreen dry Plenty of light Smaller trays Lower seed density Avoid used soil for cultivation Tray sterilization for reuse Use of biocontrol (antifungal solutions)
2.	Bacterial	Avoid unsterilized compost Seed sterilization Keep microgreen dry Plenty of light Smaller trays Air circulation Lower seed density Avoid used soil for cultivation Tray sterilization for reuse

S. No.	Pathogen	Proposed management to reduce losses due to disease
		Use of biocontrol (antibacterial solutions)
3.	Viral	Not a major threat and risk would be negligible for cross-contamination
4.	Insect pest	Use vacuum cleaner Use diatomaceous earth Use yellow sticky traps

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Innovations in Leguminous Microgreens: Recent Trends and Applications

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Abbreviations

CAGR Compound annual growth rate
CKX Cytokinin dehydrogenase
COVID-19 Coronavirus disease 2019
CRISPR Clustered Regularly Interspaced Short Palindromic Repeats
CVD Cardiovascular disease
DEHP Di-(2-ethylhexyl) phthalate
DNA Deoxyribonucleic acid
FME Fenugreek microgreen extract
GLS Glucosinolates
GR Glucoraphanin
HvZIP13 *Hordeum vulgare* Zn-regulated, iron-regulated transporter-like protein13

IL-6/10 Interleukin-6/10
LDL Low Density Lipoprotein
LED Light-emitting diode
ROS Reactive oxygen species
SFN Sulforaphane
SlNAP2 Solanum lycopersicum NAC-like activated by Apetala3/Pistillata
TALENs Transcription Activator-like Effector Nucleases
TNF-α Tumor Necrosis Factor-α
UV Ultraviolet
VLDL Very Low Density Lipoprotein
WHO World Health Organization
ZFNs Zinc Finger Nucleases

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1 Introduction

In recent years, there has been an increased interest in nutritious and organic vegetables amongst people worldwide. This interest has in turn led to the emergence of microgreens as a new popular addition to the culinary sector. Microgreens are tender and, immature vegetables which are an emerging specialty crop of the twenty-first century. These microgreens are produced from the seeds of various vegetables, herbs, and cereals as well as some wild species (Truzzi et al. 2021). When the plant is 5–10 cm tall, or before the real leaves appear, microgreens with a stem and cotyledon leaves are collected. Because they may be added to a variety of foods and drinks, including salads, sandwiches, soups, desserts, and beverages, sprouts, and microgreens have become a staple of the diets of many people (Altuner et al. 2022). Microgreens are 3–8 cm long, 7–21 days old seedlings that are mostly grown from vegetable, herb, and pulse seeds (Xiao et al. 2012). They are picked when the plants reach their first true leaf stage. They are regarded as “functional foods” and have exceptional antioxidant and nutritional qualities (Kyriacou et al. 2016). According to reports, microgreens have four to six times the amount of nutrients as their mature counterparts. This is due to the fact that during germination, structural proteins, and other cell components are synthesized concurrently with a significant breakdown of seed-storage chemicals (Xiao et al. 2012; Priti et al. 2021). Microgreens also produce minimal food waste during consumption, as no biomass (except the roots) is wasted for trimming (Weber 2017). Microgreens, unlike sprouts, require light and a growing medium, and have a longer growth cycle that varies by species. The edible section includes the stem and the first leaves. Plant phytonutrient levels vary with growth stage, with a decline observed from seedling (sprout, microgreen) to fully grown stage (Xiao et al. 2012; Priti et al. 2021).

A large number of species of plants can be consumed as microgreens. According to a study, legumes are amongst others such as cereals, oilseeds, etc. which are widely used in the production of microgreens (Wojdyło et al. 2020). Among the legumes, mung beans and lentils are considered affordable sources of microgreens as they are relatively easy to grow (Priti et al. 2021). Sprouts and microgreens are popular due to their appealing appearance and strong flavour. Secondary metabolites affect both sensory and nutritional qualities. Several investigations have found that the contents of numerous bioactive compounds accumulate considerably during sprouting and in microgreens. Polyphenols, anthocyanins, and other redox-active substances, including vitamins, glucosinolates, and minerals, have been detected in abundance. Microgreens can be biofortified with minerals

and secondary metabolites by adjusting their light regime and growth solution composition (Cigić et al. 2020).

The production of microgreens requires less time (approximately 2 weeks), is easy, and is also cost-effective which is mostly due to the requirement of simple equipment and other necessities. These suggest opportunities with the scope of industrial as well as small-scale avenues (Galieni et al. 2020). The microgreens market is quickly expanding and being marketed as a “living product” in the media. This allows customers to use them fresh as desired. Currently, the market is primarily concentrated in Western countries. In India, it is gaining popularity, particularly in major cities. In 2019, the United States contributed the most to microgreens, followed by Canada and Mexico. The global microgreens market is projected to develop at a 7.5% CAGR from 2020 to 2025. Microgreens such as broccoli, lettuce, rocket, and basil are commonly grown using hydroponics and vertical farming techniques (Charlebois 2018; Riggio et al. 2019; Priti et al. 2021).

2 Diversity of Leguminous Microgreens

Legumes are part of the Fabaceae family, which constitutes one of the most economically important plant families in agriculture. It is the third-largest family of angiosperms, containing approximately 20,000 species. They are primarily characterized by the presence of the legume fruit and their ability to symbiotically fix nitrogen.

Legumes are mainly cultivated for their edible seeds and thus are also known as grain legumes. Due to their high nutritional value and year-round production, grain legumes have become a staple in diets all over the world. Pulses, which are the dried seeds of leguminous plants, are a crucial and cheap source of protein and fibre in vegetarian diets, especially in developing countries. Some of the most economically important leguminous crops are *Arachis hypogaea* L. (peanut), *Glycine max* (L.) Merr. (soybean), *Pisum sativum* L. (pea), *Lens culinaris* Medik. (lentil), *Cajanus cajan* (L.) Huth (pigeon pea), *Cicer arietinum* L. (chickpea), *Vigna radiata* (L.) R.Wilczek (mung bean), *Phaseolus vulgaris* L. (kidney bean), *Vigna unguiculata* (L.) Walp. (cowpea), and *Medicago sativa* L.(alfalfa).

Among leguminous microgreens, pea microgreens are the most widely produced. This is because of many reasons, such as their ease of cultivation and rapid growth rate. They are also known for their vibrant flavours, crisp texture, and abundant nutritional benefits. This makes them a popular choice for cultivation both at home and in the commercial scale. Pea shoots, also known as tendril peas, are known for their sweet and earthy taste and generally adds a depth and freshness to the dish. They are used in a multitude of dishes, ranging from salads and sandwiches to stir-fries and garnishes. Rich in essential vitamins, minerals, and antioxidants, they appeal to consumers as a healthful and nutritious ingredient. Studies have found that several metabolites and minerals were higher in pea microgreens as compared to their mature counterparts (Gupta et al. 2023). Overall, the widespread popularity and versatility of pea shoots make them the most commonly cultivated leguminous microgreen. Apart from peas, other leguminous crops commonly used for microgreens production are chickpeas, lentils, alfalfa, clover, and fenugreek (Table 1).

Table 1 List of some of the most commonly cultivated legumes which can be consumed as microgreens

Scientific name	Common name	Part used from the mature vegetable	Part used as microgreen
Medicago sativa L.	Alfalfa	Leaves and Young Shoots	Stem and Leaves
Pisum sativum L.	Pea	Seeds	Stem and Leaves
Cicer arietinum L.	Chickpea	Seeds	Stem and Leaves
Lens culinaris Medik.	Lentil	Seeds	Stem and Leaves
Trigonella foenum-graecum L.	Fenugreek	Seeds	Stem and Leaves

Scientific name	Common name	Part used from the mature vegetable	Part used as microgreen
Cajanus cajan (L.) Huth	Pigeon Pea	Seeds	Stem and Leaves
Vigna radiata (L.) R.Wilczek	Mung Bean	Seeds	Stem and Leaves
Phaseolus vulgaris L.	Kidney Bean	Seeds	Stem and Leaves
Trifolium pratense L.	Red Clover	Seeds	Stem and Leaves
Glycyrrhiza glabra L.	Licorice	Seeds	Root, Stem and Leaves

3 Nutritional Benefits of Leguminous Microgreens

Leguminous microgreens are not only celebrated for their culinary appeal but also revered for their exceptional nutritional profile. They generally offer a concentrated dose of essential vitamins, minerals, antioxidants, and phytonutrients, making them a valuable addition to any diet. However, studies comparing the nutrition profile of leguminous microgreens to their mature counterparts remain limited. The following section will delve into the nutritional profiles of various leguminous microgreens that have been reported thus far (Fig. 1).

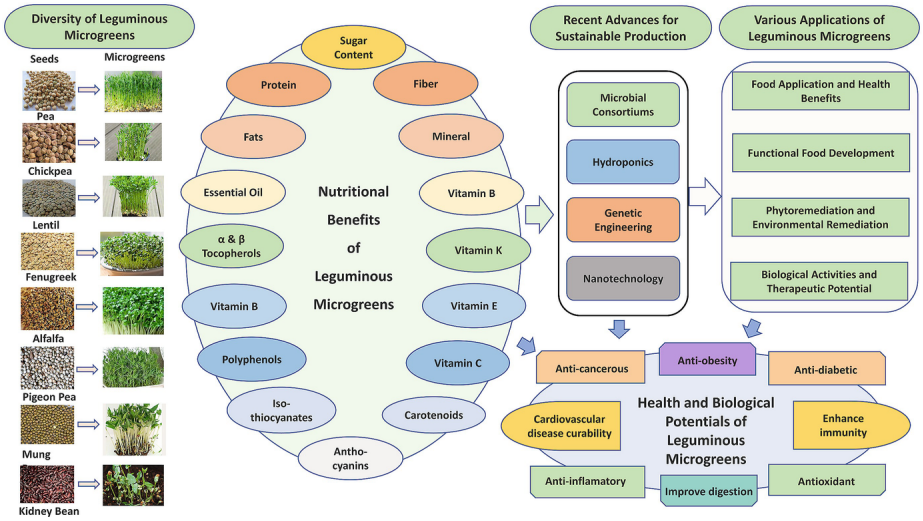


Fig. 1 Nutritional benefits of microgreens

3.1 Macro and Micronutrients

Leguminous microgreens stand out for their notable macro and micronutrient content, essential for promoting satiety, regulating blood sugar levels, and supporting muscle growth and repair. A study on seeds, sprouted seeds, and microgreens of *Trifolium pratense* L. (red clover), *T. medium* L. (zigzag clover), alfalfa, *Medicago lupulina* L. (black medick), *Onobrychis viciifolia* Scop. (sainfoin), *Astragalus glycyphyllos* L. (liquorice milkvetch) and *Astragalus cicer* L. (cicer milkvetch) found that the protein and fibre content generally increased for microgreens as compared to their raw and sprouted seed counterparts while there was a decrease in crude fat and total carbohydrate content (Butkutė et al. 2018). Fenugreek microgreens were also reported to have higher total proteins and dietary fibre contents than mature spinach, which is a common nutrient rich leafy green (Ghoora et al. 2020). Another study also reported that the most abundant elements in mungbean and lentil microgreens were in the order of potassium, phosphorus, and calcium and potassium, calcium, and phosphorus respectively. Calcium and Magnesium were significantly higher in lettuce microgreens as compared to their mature counterparts. Among the micronutrients, iron content was always recorded to be higher than zinc as reported in microgreens like *Cichorium intybus* and *Lactuca sativa*.

Different micronutrients such as iron, zinc, copper, and manganese were recorded in mungbeans in a study done in Delhi and Leh-grown microgreens (Table 2) (Priti et al. 2021). These reports suggest the superior nutritional content of microgreens when contrasted with mature vegetables.

Table 2 Essential macronutrients of microgreens

Scientific name	Total Carbohydrates (g/100 g)	Proteins (g/100 g FW)	Fats (g/100 g)	Total Dietary Fibre (g/100 g FW)	Water	References
Medicago sativa L.	29.7	44.0 ± 0.93	6.48 ± 0.21	14.5 ± 0.32	–	Butkutė et al. (2018)
Pisum sativum L.	0.36 ± 0.02	3.1 ± 0.2	0.4 ± 0.0	2.2 ± 0.1	91.6 ± 0.1	Santos et al. (2014) and Kowitcharoen et al. (2021)
Cicer arietinum L.	–	–	–	–	–	–
Lens culinaris Medik.	5.92 ± 0.01	6.47 ± 0.11	0.43 ± 0.02	–	86.57 ± 1.12	Kowitcharoen et al. (2021)
Trigonella foenum-graecum L.	5.12 ± 0.02	3.33 ± 0.06	0.18 ± 0.00	1.48 ± 0.04	90.17 ± 1.22	Ghoora et al. (2020) and Kowitcharoen et al. (2021)
Cajanus cajan (L.) Huth	–	–	–	–	–	–
Vigna radiata (L.) R.Wilczek	7.16 ± 0.02	4.55 ± 0.05	0.36 ± 0.01		87.29 ± 2.28	Kowitcharoen et al. (2021)
Phaseolus vulgaris L.	–	–	–	–	–	–
Trifolium pratense L.	26.1	45.7 ± 0.43 bcA	4.84 ± 0.23 bcB	17.5 ± 1.06 aA	–	Butkutė et al. (2018)
Glycyrrhiza glabra L.	–	–	–	–	–	–

3.1.1 Vitamins and Minerals

Microgreens of different legumes viz., *Trifolium medium, T. pratense, Medicago lupulina, M. sativa, Onobrychis viciifolia, Astragalus cicer*, and *A. glycyphyllos* have been reported to possess higher concentrations of several minerals like potassium, phosphorus, and zinc as compared to the raw and sprouted seeds (Butkutė et al. 2018). Researchers while conducting a study on the elemental composition of fenugreek microgreens found that they had higher nutritional value as compared to mature spinach leaves. Studies on vitamin compositions led to the conclusion that fenugreek microgreens had 120% more ascorbic acid, which is an essential micronutrient for the human body, compared to their mature counterparts. Similarly, alpha tocopherol which is a biologically active form of vitamin E was found to be over 14-folds higher in fenugreek microgreens than in mature fenugreek (Table 3) (Longvah et al. 2017; Butkutė et al. 2018). In another study, pea microgreens were also reported to have uniquely high alpha tocopherol content compared to other microgreens, thus making them a potential source of vitamin E (Johnson et al. 2021). Interestingly, the sodium content however was found to be low in various microgreens such as pea tendrils. Microgreens have the potential to be superfoods with high levels of various desirable nutrients while also being low in undesirable nutrients like sodium (Di Gioia et al. 2017).

Table 3 Minerals of Leguminous Microgreens

Scientific name	Na (mg/100 g)	Ca (mg/100 g)	Mn (mg/100 g)	Cu (mg/100 g)	Mg (mg/100 g)	K (mg/100 g)	Zn (mg/100 g)	Fe (mg/
Medicago sativa L.	–	0.611 ± 0.020	–	–	0.373 ± 0.069	1.49 ± 0.01	27.3 ± 1.69	17.8
Pisum sativum L.	6.46 ± 0.22	58.17 ± 2.92	0.33 ± 0.01	0.12 ± 0.00	27.84 ± 0.92	332.4 ± 6.02	0.49 ± 0.01	1.48
Cicer arietinum L.	–	–	–	–	–	–	–	–
Lens culinaris Medik.	45 ± 3.61	70 ± 5.29	0.18 ± 0.035	0.22 ± 0.028	66 ± 4.58	369 ± 11.79	0.49 ± 0.015	0.79
Trigonella foenum-graecum L.	52.0 ± 0.9	57.7 ± 5.3	–	–	17.9 ± 0.1	118.5 ± 1.0	0.94 ± 0.02	1.00
Cajanus cajan (L.) Huth	–	–	–	–	–	–	–	–
Vigna radiata (L.) R.Wilczek	45 ± 3.61	86 ± 2.6	0.019 ± 0.0013	0.10 ± 0.0009	56 ± 2.60	369 ± 14.9	0.32 ± 0.019	0.76
Phaseolus vulgaris L.	–	–	–	–	–	–	–	–
Trifolium pratense L.	–	0.238 ± 0.012 eC (g/100 g)		–	0.358 ± 0.016 bC (g/100 g)	1.34 ± 0.06 cdA (g/100 g)	29.9 ± 1.50 aA	16.5 bcA
Glycyrrhiza glabra L.	–	–		–	–	–	–	–

3.2 Bioactive Compounds

Studies have found that species of clover microgreens (red and zigzag clover) had higher concentrations of isoflavones as compared to raw and sprouted seeds (Butkutė et al. 2018). The same study also found higher coumestrol and saponins in alfalfa microgreens as compared to their raw and sprouted seed counterparts. Pea microgreens have been reported to contain some unique compounds like kaempferol glycosides and trifolin, a kaempferol galactoside, which are not normally found in their mature counterparts (Johnson et al. 2021). Glycosylflavones, reportedly possessing medicinal properties like xanthine oxidase inhibition, were also isolated from fenugreek microgreens, some of which were reported for the first time in the genus (Guo et al. 2023). This study thus suggests how unique compounds isolated from microgreens might also have medicinal value. Polyamines like agmatine, spermine, and spermidine which are beneficial nutritionally were found to have accumulated in higher concentrations in lentil, fenugreek, and alfalfa microgreens than sprouts (Cigić et al. 2020). These findings suggest the potential of leguminous microgreens as valuable sources of essential nutrients such as macronutrients, vitamins, minerals, and phytochemicals. However, further comprehensive nutritional profiling studies are needed to fully comprehend their nutritional benefits and optimize their incorporation into people's diets.

4 Recent Advances for Sustainable Production

With the current climate change scenario, sustainable agriculture now stands as one of the most promising avenues for a greener future. In pursuit of this, several techniques like the use of microbial consortiums, genetic engineering and nanotechnology have made it possible to produce microgreens in an eco-friendly manner. The following section will discuss the potential of these technologies in enhancing the production of leguminous microgreens.

4.1 Microbial Consortiums

With the rising criticism of chemical fertilizers various alternatives have been developed for sustainable agriculture. Among the various new alternatives, utilization of beneficial microbes has gained substantial momentum mainly due to its eco-friendly approach. Utilising microorganisms to increase crop nutrient accessibility and thus enhance soil nutrient status is a form of green technology (Dhaliwal et al. 2022). Beneficial microbes residing in the rhizosphere—the soil region surrounding plant roots offer a variety of benefits, including promoting plant growth, suppressing diseases, detoxifying soil, and facilitating nutrient uptake by plants (Vishwakarma et al. 2020). By a variety of processes, including synthesis, mobilisation, transformations, and siderophore synthesis, soil microorganisms increase the amount of nutrients in the rhizosphere and help plants accumulate more minerals (Dhaliwal et al. 2022). *Bradyrhizobium* sp. has been reported to increase phosphorus and nitrogen uptake by soybeans (Fituma et al. 2017). The application of *Pseudomonas* and *Bacillus* sp. decreased soil pH, releasing zinc ions from complex compounds. *Arthrobacter* sp. and *Arthrobacter sulfonivorans* enhanced the Zn and Fe levels in wheat. Additionally, combining *Bacillus subtilis* with *Arthrobacter* sp. and other endophytes boosted the yield and micronutrient status in wheat (Singh et al. 2018). Soil inoculated with mycorrhizal fungi increases micronutrient availability due to siderophore synthesis, rhizospheric acidity, and hyphal transfer of nutrients through the external mycelium (Dhaliwal et al. 2022). In another study, it showed that when *Rhizophagus irregularis* colonised under Zn-deficient conditions, higher grain Zn concentrations were seen due to HvZIP13 overexpression (Watts-Williams and Cavagnaro 2018). In Inoculation of *Phaseolus vulgaris* (common bean) with *Cellulosimicrobium funkei* was found to increase seed germination, shoot, and root length, and biomass as well as photosynthetic pigments like chlorophyll and carotenoids (Karthik et al. 2016). The same study also found that *C. funkei* inoculation led to decreased Cr (VI) accumulation in the plant thus suggesting its use in metal contaminated soils. Inoculating *Fagopyrum esculentum* (buckwheat) microgreens and sprouts with the bacterial endophyte *Herbaspirillum* sp. was reported to enhance its growth rate (Briatia et al. 2017). Similarly, using *Pseudomonas* sp. consortia based biofertilizer was seen to significantly enhance growth in broccoli microgreens (Guardiola-Márquez et al. 2023). While research concerning the utilization of beneficial microbes for leguminous microgreen production remains absent, the above reports suggest the potential use of microbial consortia as biofertilizers for their sustainable production.

4.2 Hydroponics

Hydroponics is the practice of growing plants without soil by using nutrient-rich solutions. This soil-free agricultural technique has many advantages like increased nutrient levels, cleaner growing conditions, and quicker harvesting schedules. Additionally, hydroponic systems require less room and water, which makes them perfect for indoor farming in places with limited land. Research shows that hydroponically grown plants consume about 10% of the water used by the conventionally farmed plants making them a more efficient choice for agricultural water management in regions with water scarcity issues (Rorabaugh et al. 2002). The soilless cultivation of microgreens has gained recognition recently for its ability to combat deficiencies likely due to their rich nutritional profile and enhanced flavors (Xiao et al. 2012; Kyriacou et al. 2016). Hydroponically grown microgreens from the Brassicaceae family have been successfully fortified with micronutrients like selenium, zinc and iron (Puccinelli et al. 2019; Pannico et al. 2020). Similarly, hydroponically grown *Daucus carota* L. (carrot) and *Anethum graveolens* L. (dill) microgreens combined with protein hydrolysates were found to have significantly high concentrations of total proteins and amino acids, suggesting the potential role of hydroponics in biofortification (Di Gioia et al. 2019; El-Nakhel et al. 2021). A study also found that hydroponically grown *Ocimum basilicum* L. (basil), *Beta vulgaris* L. (Swiss

chard), and *Eruca sativa* Mill. (arugula) microgreens had low concentration of nitrate compared to their mature or baby leaf counterparts (Bulgari et al. 2017). This hints that using hydroponics to grow microgreens could be a key technique in to increase the concentration of desired nutrients while keeping undesired nutrient concentrations to a minimum. Additionally, hydroponics has emerged as a method for treating wastewater by allowing plants to absorb contaminants from the water before its discharge (Cifuentes-Torres et al. 2020). Wastewater was also found to be a suitable growing medium for edible microgreens, thus making them a sustainable method for water recycling (Selvan and Salvadeeswaran 2021). Since hydroponics involves growing crops in a controlled environment, it enables crop production throughout the year. The growing medium plays a big role in the cost of food production and since there is no dependence on seasonal variations and weather conditions, food production and prices are also stabilized. This results in higher productivity and a more stable supply chain (Rajaseger 2023). Diseases and pests which are usually transmitted through the soil can also be limited on hydroponically grown microgreens (Du et al. 2022). Hydroponics thus stands as a method that will guarantee a regular and plentiful supply of microgreens while at the same time addressing sustainability and water use issues.

4.3 Genetic Engineering

Genetic engineering provides many options to enhance crop resilience, nutritional value, and yield efficiency and hence is currently recognized as an important technique in sustainable agriculture (Datta 2013). One major downside of microgreens is their limited shelf life. Storage at lower temperatures, such as 4.44 °C or 10 °C, extends shelf life, with durations ranging from 7 to 21 days for various microgreens. Specific temperature ranges, such as 1.6–4.44 °C or ambient temperatures of 5 °C, also influence shelf life, varying from 13 to 28 days for different types of microgreens (Sharma et al. 2023). Effective storage systems and chemical treatments, like calcium lactate or chlorine, have been used to enhance shelf life. In this milieu, genetic engineering could be a more sustainable way to increase the shelf life of microgreens. Silencing the genes involved in senescence may allow us to delay the beginning of senescence and improve biomolecule preservation for a longer period (Sharma et al. 2022). Inhibition of the SlNAP2 transcription factor in tomatoes was found to postpone senescence while boosting sugar content and fruit yield, most likely due to the plants' prolonged photosynthetic period (Ma et al. 2018).

Biofortification is the process of enhancing the nutritional value of plant edible parts, offering a sustainable solution for providing people with micronutrient-rich crops. It involves improving vitamins, amino acids, minerals, and fatty acids while reducing anti-nutritive factors and addressing malnutrition and hidden hunger. In the face of climate change impacting crop yield and quality, biofortification emerges as a crucial strategy, especially for developing nations. It can be achieved through means like genetic engineering, and OMICS approaches to develop genotypes with improved traits like improving breeding cycles and overall nutrient content for sustainable food production. Genetic manipulation techniques such as Agrobacterium-mediated transformation, electroporation, and particle gun bombardment have been utilized to introduce desirable traits, including tolerance or resistance to various stresses, in plants. These transgenic plants can exhibit numerous desired traits like insect pest resistance, disease resistance (fungal, viral, and bacterial), and enhanced production of compounds like carotenoids and flavonoids through modification of metabolic pathways. Multi-trait transgenic plants can be developed using in vitro gene stacking systems. Chickpeas have been targeted for improvement by manipulation of the cytokinin dehydrogenase (CKX) gene by using genome editing technology has also been reported to improve nutritional parameters in chickpeas (Mahto et al. 2022).

Microgreens are naturally nutrient-dense crops rich in vitamins, polyphenols, and glucosinolates, offering health benefits and antioxidant properties without necessarily requiring biofortification (El-Nakhel et al. 2021). Nevertheless, the application of these tools can further enhance their nutritional value, thus making them an even better superfood. The identification and breeding of potential microgreen genotypes, as well as the biofortification and, integration of other desired traits such as faster growth, enhanced shelf life, etc, can be achieved by an integrated approach comprising various techniques of genetic engineering, genome editing, foodomics, and nutriomics. However, one of the major drawbacks of this is the public's skepticism surrounding the health risks and effects of consuming genetically modified crops. Although these myths have been debunked time and over again, general acceptance of GMOs remains truncated (Bawa and Anilakumar 2013; Lucht 2015). Plant genome editing has been reported to utilize sequence-specific nucleases like ZFNs, TALENs, and CRISPR-Cas9 to modify genes in crops, aiming to create transgene-free plants and enhance traits like shelf life; recent efforts include biofortification in chickpeas for improved nutrition (Gupta et al. 2023).

4.4 Nanotechnology

Microgreens production is also possible using nanotechnology. It is an area of research that involves dealing with materials at the nanoscale level ranging from 1 to 100 nm. Due to its precision at the atomic and molecular level, this technology can provide an unparalleled potential to revolutionize various research sectors ranging from medicine to electronics. It involves the use of various nanosized matter like nanoparticles, nanofibers, etc. In our currently studied area of microgreens production, it can be a boon in the form of nano fortification which involves fortifying plants or microgreens with various essential nutrients such as copper, selenium, iron, and zinc. This can be done in the form of liquid treatment directly onto the leaves, or by using nano fertilizers in either soil or water mediums (El-Ramady et al. 2021). For example, using of ZnO and γ-Fe_2O_3 nanoparticles functionalized with a *Pseudomonas* species preparation as nanofertilizers was shown to enhance plant growth and improve the micronutrients and glucosinolates content in broccoli microgreens (Guardiola-Márquez et al. 2023). Nanotechnology can also help in microgreens production by the use of nanoparticles for seed priming. A study was done to see if seed priming could be carried out with nanoparticles (Rhaman et al. 2022). It led to an increase in germination activity and nutrient uptake. Through our literature review, we also found a study that concluded that the use of iron oxide NPs has led to enhanced germination, shoot length, and other growth parameters (Sundaria et al. 2018).

Recent advances in nanotechnology have significantly contributed to the sustainable production of microgreens. One notable innovation is the development of biodegradable and eco-friendly nano-coatings for seeds, which enhance seedling vigor and protect against pathogens without leaving harmful residues in the soil (Mittal et al. 2020; Elemike et al. 2019; El-Ramady et al. 2021). These nano-coatings are designed to gradually release nutrients, thereby ensuring a steady supply of essential elements during the critical stages of germination and early growth (Shang et al. 2019; Nile et al. 2022). Additionally, the integration of nanosensors in microgreens cultivation offers real-time monitoring of soil moisture, nutrient levels, and plant health, enabling precise irrigation and fertilization practices that minimize resource wastage and environmental impact (Bombin et al. 2015). These advancements not only enhance the nutritional profile and growth efficiency of microgreens but also align with the principles of sustainable agriculture by reducing the reliance on conventional fertilizers and pesticides. As research continues to evolve, the integration of nanotechnology in microgreens production holds great potential for achieving more resilient and sustainable agricultural systems.

5 Various Applications of Leguminous Microgreens

5.1 Food Application and Health Benefits of Leguminous Microgreens

Leguminous microgreens are emerging as versatile ingredients with diverse applications in the food and pharmaceutical industries. These tiny greens boast high levels of essential vitamins, minerals, proteins, dietary fibers, and bioactive compounds, such as total anthocyanins, polyphenols, ascorbic acid, and isothiocyanates as discussed in the previous section, thus exhibiting health-promoting potential and therefore have often been referred to as superfoods and functional foods (de la Fuente et al. 2019; Martiniakova et al. 2022). Adequate intake of essential nutrients, including macro and oligo-elements like potassium, calcium, magnesium, iron, selenium, copper, and zinc, is crucial for proper growth, metabolism, and immune system function (al-Bader et al. 1998). In this line, microgreens from various sources, have been found to be rich in minerals and nutritionally dense components compared to their mature counterparts (Johnson et al. 2021). The nutritional quantities of these microgreens are influenced by their growing conditions and genotype (Kyriacou et al. 2021; Tan et al. 2020), however, harvesting time has been reported to have no influence (Kyriacou et al. 2021).

Leguminous microgreens add not only vibrant colors but also distinct flavors and textures to various dishes, ranging from salads to soups (Xiao et al. 2015). Their aesthetic appeal makes them popular choices for garnishes and decorations, elevating the visual presentation of culinary creations. These microgreens because of their versatile nature can also be incorporated into other staples to enrich and complement their nutritional composition. For instance, Klopsch et al. (2018), developed bread enriched with leguminous microgreens and leaves.

Beyond their food application, leguminous microgreens hold promise in pharmaceutical applications due to their abundance of bioactive compounds, including antioxidants and anti-inflammatory agents. Its nutritional composition is cited to have immunity-regulating properties and contribute to combating various health threats including osteoporosis, pharmacotherapy, and diseases like COVID-19 (Kyriacou et al. 2019; Xiao et al. 2012).

Studies are emerging that uphold the health potential of these microgreens. Truzzi et al. (2021) studied the effects of different light conditions on pea microgreens. They reported that pea microgreens grown under LED light demonstrated anti-proliferative and pro-apoptotic effects on cancer cell lines without harming healthy cells. Additionally, Truzzi et al. (2021) also reported that non-leguminous microgreens like Red Rambo radish illuminated by LED and fluorescent light also reported to exhibit anti-tumor effects. Further research on the influence of UV light on microgreens' polyphenol content and anti-tumor activities could provide valuable insights.

As interest in natural and functional foods grows, leguminous microgreens are poised to play an increasingly significant role in food and pharmaceutical industries owing to their nutritional superiority, sensory parameters, and bioactive compounds.

5.2 Culinary Applications and Functional Food Development

As discussed in the earlier sections, microgreens are a promising functional food crop with high nutritional quality, low environmental impacts, and broad consumer acceptance, making them suitable for diversifying global food systems and promoting human health. Microgreens offer a spectrum of culinary possibilities, enriching salads, shakes, sandwiches, sweets, savory dishes, flatbreads, pizzas, and soups with their nutritional properties (Fig. 2). Their high protein content and abundance of essential antioxidants, minerals, and vitamins make them suitable for inclusion in functional foods. Also, microgreens present a spectrum of flavors, vibrant colors (such as red, purple, green, and yellow), varied appearances, and textures, ranging from tender and juicy to crisp making them suitable decorative elements in various dishes (Sehrish et al. 2023).

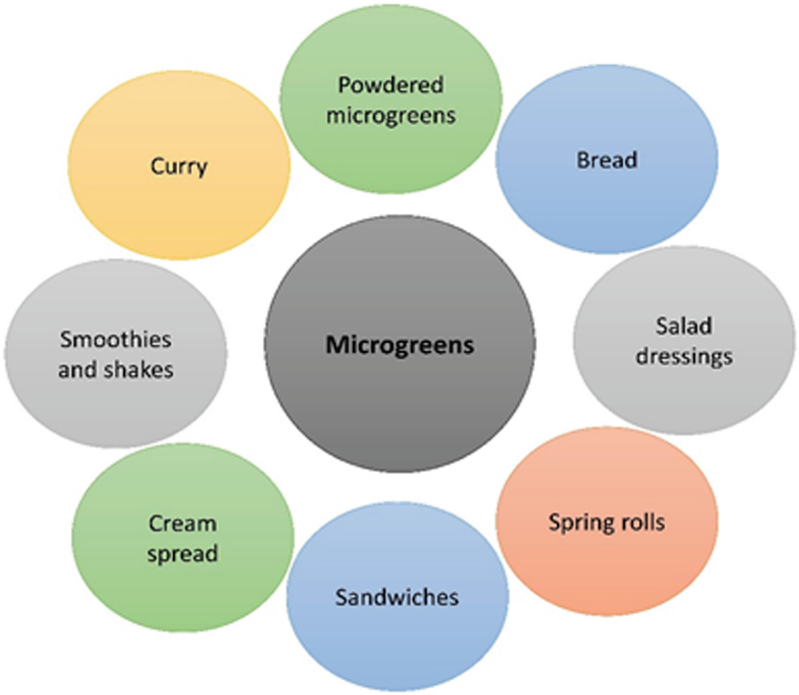


Fig. 2 Microgreens in culinary application

Microgreens offer opportunities for innovation in food products, including beverages, probiotic-infused products like yogurt, flour enriched with powdered microgreens, seasonings, bakery items, and teas (Aminah et al. 2019; Islam et al. 2019). Simsek et al. (2014) incorporated microgreens and sprouts of lentils and cowpeas into vegetable and fermented vegetable juice, thus enriching its nutritional value. Similarly, Bello et al. (2018) used broccoli sprouts to prepare juice. Broccoli sprouts are recognized as functional foods due to their high glucoraphanin (GR) content and are valued for their potential to produce sulforaphane (SFN), an anticancer compound. Such products enriched with bioactive compound-containing ingredients demonstrate various beneficial effects.

Gawlik-Dziki et al. (2017) reported that microgreens and leaves from Brassica species like pak choi and kale contain bioactive substances, including breakdown products of glucosinolates (GLS), carotenoids, chlorophyll metabolites, and phenolic compounds, which offer a range of health benefits. In their study, they incorporated sprouts and microgreens into bread, which enhanced their nutritional value significantly, offering antiradical and chelating compounds and phytochemicals that protect lipids against oxidation. Incorporating these microgreens into common staple dietary items enhances the diet's bioactive, health-promoting substances.

In a similar line, Hidalgo et al. (2019), worked with two cereal microgreens, wheat, and barley, and incorporated them into biscuits. They found that incorporating 15–20% sprouted wheat or 5–10% sprouted barley into biscuits significantly improved their nutritional profile. This enrichment increased levels of beneficial compounds such as carotenoids, tocols, and phenolics, while also reducing heat damage effects. This highlights the potential of using sprouted grains to enhance the health benefits of baked goods.

Likewise, Falcinelli et al. (2018) and Hernandez-Aguilar et al. (2020) also used wheat and lentil sprouts respectively, to enrich bread. These functional products exhibit antioxidant potential and resistance to high temperatures, making them valuable additions to functional foods.

Pannico et al. (2022) investigated the feasibility of producing Hemp (*Cannabis sativa* L.), a versatile microgreen crop. They evaluated six cultivars for yield and composition of organic acids, amino acids, polyphenols, and phytocannabinoids. Despite its numerous health benefits (Leonard et al. 2020), the potential use of hemp vegetative tissue as a novel food source has been largely overlooked. The bioactive composition varied significantly among hemp varieties, with 'Silvana' having high amino acid content and 'Finola' containing high levels of cannflavins and polyphenols.

García-Tenesaca et al. (2024) investigated the influence of selenium enrichment in microgreens like green pea, red radish, and alfalfa. They found that concentrations of 20 µM led to significant Se biofortification without negatively impacting phenolics and antioxidant capacity in green pea and alfalfa. However, red radish showed a decrease in these compounds. Se treatment increased chlorophylls and carotenoids in green pea, decreased chlorophyll levels in alfalfa, and did not affect red radish. Their study indicated that incorporating Se-biofortified microgreens into the diet could be a practical strategy to enhance overall selenium intake and potentially improve health outcomes related to selenium deficiency.

5.3 Phytoremediation and Environmental Remediation

Phytoremediation, the use of plants to remediate environmental pollutants, has gained prominence due to its eco-friendly and sustainable nature. Microgreens have emerged as promising candidates for phytore mediation due to their rapid growth and efficient nutrient uptake systems. Several studies have demonstrated the ability of microgreens to remediate various environmental pollutants, including heavy metals and organic contaminants. Testa et al. (2023) investigated the phytoremediation potential of several microgreens for heavy metals in contaminated soils, revealing significant accumulation of metals such as cadmium and lead in the shoots (Alsafran et al. 2022). Haller et al. (2023) investigated the remediation of petroleum hydrocarbons by microgreens, demonstrating a significant reduction in soil contamination following cultivation. Furthermore, various research conducted reviews highlighting the potential of microgreens for the remediation of heavy metals in soil, emphasizing their role in soil restoration and pollution mitigation. These studies underscore the versatility of microgreens in addressing various environmental contaminants and their potential contribution to ecosystem health (Butkutė et al. 2018; Teng et al. 2021; Gao et al. 2021).

Legume microgreens, derived from plants such as *Pisum sativum* (pea), *Cicer arietinum* (chickpea), *Lens culinaris* (lentil), *Trigonella foenum-graecum* (fenugreek), *Medicago sativa* (alfalfa), *Cajanus cajan* (pigeon pea), *Vigna radiata* (mung bean), and *Phaseolus vulgaris* (bean), have garnered attention for their potential in phytoremediation and environmental restoration. While specific studies on the phytoremediation capabilities of microgreens from these species are limited, research on their mature counterparts provides valuable insights. For instance, peas have been studied for their ability to uptake heavy metals from contaminated soils (Thuong and Minh 2020; Medyńska-Juraszek et al. 2022), while chickpeas contribute to soil fertility and erosion control (Luo et al. 2017). Lentils have shown promise in enhancing soil structure and water retention, and fenugreek has been explored for its potential in heavy metal remediation (Rodriguez et al. 2007; Dheri et al. 2007; Niu et al. 2017; Kaur 2018; Nandi et al. 2021). Alfalfa, known for its deep root system, and nitrogen-fixing abilities of pigeon peas also offer potential avenues for environmental remediation (Teng et al. 2021; Chen et al. 2022). The collective characteristics of these legume microgreens, including rapid growth, efficient nutrient uptake, and adaptability to diverse environmental conditions, therefore suggest their suitability for phytoremediation and environmental restoration. While further research is needed to specifically assess the phytoremediation potential of microgreens derived from these legumes, their mature counterparts' capabilities provide a foundation for exploration. Integration of legume microgreens into phytoremediation strategies holds

promise for addressing environmental challenges, offering sustainable solutions for pollution mitigation and ecosystem restoration.

5.4 Biological Activities and Therapeutic Potential

Microgreens are rich in phytochemicals, including sulphur compounds (glucosinolates and their derivates—isothiocyanates), flavonoids (i.e. isoflavones, flavanols, anthocyanins), phenolic acids, lignans, carotenoids, betacyanins, or stilbenes (Grudzińska et al. 2023). Many of these phytochemicals possess bioactive properties and can have pharmaceutical applications. Some of the therapeutic applications of microgreens have been discussed in this section.

5.4.1 Antioxidants

Imbalances in production of reactive oxygen species (ROS) and antioxidant defence mechanisms in the body can lead to oxidative stress, which over time causes to oxidative damage to lipids, proteins, and DNA. Such damages lead to cellular dysfunction and may contribute to the advent of various age-related diseases, such as Alzheimer's disease, cardiovascular diseases, diabetes, and cancer. Leguminous microgreens contain considerable amounts of several phytochemicals, including flavonoids, phenolic acids, and carotenoids. Although it has been suggested that these phytochemicals are responsible for the antioxidant activities, further studies might be required to establish a correlation between the amounts of these phytochemicals to the total antioxidant activity. A 2022 study made on various leguminous microgreens has shown that among leguminous plants, red clover shows the highest antioxidant activity with a very highly phenolic content. Although this could be indicative of a direct correlation between antioxidant activity and total phenolic content, data is not sufficient for a conclusive correlation. Alfalfa, mung bean and lentils have also shown significant antioxidant activities (Altuner et al. 2022).

5.4.2 Anticancer Properties

Cancer is responsible for more than ten million annual deaths (Kocarnik et al. 2022) and is the second-leading cause of death, second only to cardiovascular diseases (Siegel et al. 2021). Owing to their rich bioactive compound content, microgreens have been recommended as functional food in prevention against various forms of cancer. While microgreens of the Brassicaceae family are more widely studied, studies are now being made on leguminous microgreens as well. Green pea microgreens, for instance, have shown potent anti-cancerous properties. Green pea microgreen extracts significantly reduced cell proliferation of Ewing sarcoma RD-ES and A673 cell lines, both in 2D and 3D cultures, with no cytotoxic effects on healthy L929 fibroblast cells (Truzzi et al. 2021). Thus, incorporation of microgreens in one's diet could help in reduction of the risk of cancer.

5.4.3 Anti-diabetic Properties

International Diabetes Federation reports suggest that 537 million people worldwide are affected with diabetes (IDF 2021). Majority of these cases are type 2 diabetes, and type 1 diabetes only accounts for about 10% of these cases. While preventive measures for type 1 diabetes are not known, type 2 diabetes can be prevented or delayed by maintaining an active lifestyle and a healthy diet (Alustiza et al. 2021). As leguminous microgreens are rich in phenolic content, flavonoids, and antioxidants, their consumption may contribute to control of diabetes. For instance, antidiabetic effects have been reported in fenugreek microgreen when in vitro studies were made using HepG2 and L6 cells. Fenugreek microgreen extract (FME) has been shown to inhibit α-amylase in a concentration-dependent manner (IC50 = 1 mg mL−1), with a maximum achievable inhibition of 70%. An FME concentration of 10 mg mL−1 also enhances the uptake of glucose by L6 cells by 25%, which may be improved to 40% in presence of insulin (Wadhawan et al. 2017).

5.4.4 Anti-inflammatory Properties

Inflammation is a protective response of the body against certain harmful stimuli, such as pathogens, damaged cells and irritants. The main function of inflammation is neutralization of the stimuli. Distinction between foreign stimulant and healthy cells is essential, and errors in such distinction can lead to inflammation against self, causing autoimmune diseases. Moreover, chronic inflammation may lead to manifestation of various diseases, such as hay fever, periodontal disease, atherosclerosis, and osteoarthritis. In such cases, anti-inflammatory agents may help in alleviating the inflammation. Classical studies on licorice extract have reported high amounts of secondary metabolites which have antimicrobial, antiviral, antitumor, antidiabetic, anti-inflammatory, immunoregulatory, hepatoprotective, and neuroprotective activities. Studies on root extracts from licorice microgreens have shown inhibitory effects on the pro-inflammatory cascade, thereby mitigating cytotoxic effects. Licorice extracts have shown protective effects on the proliferation and viability of Caco-2 cells after pro-inflammatory induction of lipopolysaccharides, with significant reduction in inflammatory markers, such as TNF-α, IL-6, and IL-10 (Marotti et al. 2021).

5.4.5 Cardiovascular Diseases

Cardiovascular diseases account for more than 17 million deaths each year and is the leading cause of death around the world (except in Africa) (WHO 2011). An estimated 53% of CVD deaths and 57% of CVD disabilities are associated with dietary risk factors (Petersen et al. 2021) and increasing vegetable and fruit intake has been shown to significantly lower the risk of cardiovascular diseases (Tayyem et al. 2020). Studies have already been made on the efficacy of non-leguminous microgreens in preventing cardiovascular diseases. Consumption of red cabbage microgreens, for instance, has been shown to lower the levels of low-density lipoprotein and hepatic cholesterol ester in mice with high-fat diet-induced obesity (Huang et al. 2016). Moreover, it has been established that consumption of mung beans helps in regulating lipid metabolism. Studies have shown significant reduction in cholesterol, triglycerides, VLDL (Very Low Density Lipoprotein) and LDL (Low Density Lipoprotein) levels in hyperlipidemic rats fed with mung bean extracts (Ganesan and Xu 2018). These results are indicative of the potential of leguminous microgreens in prevention of CVDs, although further analysis is required as the chemical contents in the microgreens may vary from those in the seeds.

5.4.6 Other Therapeutic Applications

Other than the ones mentioned above, leguminous microgreens may have several other health benefits. Flavonoids have been known to improve the biological activities of the gut microflora (Sharma et al. 2022). The high flavonoid content in leguminous microgreens, along with the high fiber content, aids in proper digestion and can promote intestinal health. The high iron content in red clover and alfalfa can also help tackle iron deficiency (Butkutė et al. 2018).

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6 Certain Issues Related to Leguminous Microgreens

Microgreens, recognized for their concentrated nutritional profiles and versatile culinary applications, are subject to various challenges that warrant careful consideration in both production and consumption. One significant issue is the risk of microbial contamination during cultivation and handling, as microgreens are typically grown in moist environments ideal for microbial growth. This can increase the likelihood of contamination by foodborne pathogens such as *Salmonella* and *E. coli*, posing potential health risks to consumers (Caleb et al. 2013). Additionally, microgreens have a short shelf life due to their high metabolic activity and delicate nature, leading to rapid deterioration in quality and nutrient content. This limitation necessitates careful handling and storage

to maintain freshness and minimize waste, particularly in commercial distribution chains where extended shelf life is desirable (Renna and Paradiso 2020; Gupta et al. 2023). Moreover, the cultivation of microgreens can be resource-intensive, particularly in indoor production systems. Riggio et al. (2019) highlight the significant inputs of water, energy, and labor required for successful microgreens cultivation, raising concerns about the environmental sustainability of large-scale production. Additionally, microgreens have the potential to accumulate heavy metals from the environment, depending on growing conditions and substrate quality. This poses risks to human health if consumed in large quantities, emphasizing the importance of monitoring growing conditions and substrate quality to mitigate contamination risks (Abaajeh et al. 2023; Gupta et al. 2023).

Despite these challenges, ongoing research into cultivation practices, food safety measures, and alternative production methods may help address these issues and unlock the full potential of leguminous microgreens as a nutritious and sustainable food source. Further studies are needed to develop effective strategies for minimizing microbial contamination, extending shelf life, and reducing resource inputs in microgreens production. Additionally, comprehensive risk assessments and regulatory guidelines are essential to ensure the safety and quality of leguminous microgreens for consumers (Riggio et al. 2019; Choe et al. 2018).

7 Conclusion and Future Perspectives

There is an increasing interest in leguminous microgreens emphasizing on their capacity as valuable contributors to both the culinary and nutritional sectors. These young tender greens are not only rich in both taste and texture but also have exceptional nutritional profiles. It is observed that the nutrient density of these microgreens is often more than that of the mature plants. Moreover, the wide range of bioactive compounds contained in the leguminous microgreens such as polyphenols, vitamins, minerals, and phytochemicals establish their status as functional foods with potential health benefits. However, extensive research is still needed to further improve the production methods of leguminous microgreens. For instance, it is necessary to carry out comprehensive nutritional profiling studies to understand fully the range and bioavailability of various bioactive compounds present in different varieties of microgreens. This will not only help us know about the dietary requirements of humans but also the cultivation practices optimized for maximizing their nutritional value.

Additionally, with the progress in sustainable farming methods, promising ways to meet the growing demand for microgreens while minimizing environmental impact are highly expected. Hydroponics, microbial consortia, genetic engineering, and nanotechnology are some of the technologies that hold the potential to enhance the yield, nutrition, and shelf life of microgreens. By integrating these innovative approaches into microgreens cultivation, we can help boost sustainability efforts in agriculture and ensure a steady supply of fresh, and nutrient-rich greens for consumers globally. Further collaborations among researchers, food scientists, and growers will be crucial for driving innovation and unlocking the full potential of leguminous microgreens. This shall not only ensure nutrition security in the current scenario but shall ensure nutrition security for future generations as well.

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Advancing Cultivation Substrates and Methods to Elevate Nutritional Quality and Production of Leguminous Microgreens

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Abbreviations

Ca Calcium

CEA Controlled Environmental Agriculture

Fe Iron

GMP Growing Mix Particles

K Potassium

LED Light-Emitting Diode

Mg Magnesium

N Nitrogen

Na Sodium

S Sulfur

Se Selenium

Zn Zinc

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1 Introduction

In recent years, microgreens have emerged as a nutritional powerhouse, captivating the attention of health-conscious consumers and culinary enthusiasts alike. It represents a burgeoning category of food item derived from harvesting the aerial portions of young seedlings of various plant species. The commonly cultivated microgreens are amaranth, basil, beet, cabbage, celery, chervil, Chinese kale, cilantro, fennel, mustard, parsley, radish, and swiss chard, offering a diverse range of flavours and textures (Pinto et al. 2015; Samuolienė et al. 2013; Brazaitytė et al. 2013, 2015; Kou et al. 2013; Bhaswant et al. 2023). They have the potential to enhance economic, food, and

nutritional security, particularly in regions facing challenges such as “hidden hunger” (Samuolienė et al. 2013; Bhaswant et al. 2023; Yeargin et al. 2023). Recently, wild edible species have also been incorporated into this category (Kyriacou et al. 2016; Palmitessa et al. 2020). Among these miniature greens, leguminous microgreens stand out for their exceptional nutritional profile and culinary versatility. Grain legumes play a crucial role in meeting human dietary protein nitrogen (N) requirements, contributing approximately 33% of the needed protein nitrogen (Vance et al. 2000). Additionally certain legume microgreens exhibit lower levels of antinutrients such as phytic acid and tannins compared to their seeds.

The pressing need to address nutritional deficiencies is underscored by global hunger indices, emphasizing the importance of ensuring widespread access to highly nutritious food for all segments of society, especially in countries like India, experiencing significant challenges in this regard (von Grebmer et al. 2022). Microgreens are promising noted for their high levels of bioactive compounds such as polyphenols, carotenoids, vitamins (C, E, and K, beta-carotene), tocopherols, and other antioxidants, which have garnered interest among consumers seeking to integrate novel, healthful, and functional foods into their diets (Di Gioia et al. 2017a; Pinto et al. 2015; Amitrano et al. 2023; Butkutė et al. 2018).

A suitable growing medium is indispensable for the development of microgreen (Du et al. 2022). They are well-suited for indoor and greenhouse cultivation owing to their compact size, robust nutritional profile, and independence from external fertilization due to their short growth cycle. In response to consumer trends favouring health-oriented foods, the agricultural sector is innovating agronomic methods to enhance microgreen quality while reducing production costs. Enhancing cultivation substrates and methods is pivotal for optimizing microgreen growth across

various fronts. This improvement facilitates maximum nutrient uptake, ensuring microgreens boast rich reserves of essential vitamins, minerals, and antioxidants, thereby upholding their reputation for superior nutrient density compared to mature plants. Additionally, it fosters expedited and uniform growth, critical for commercial growers striving to augment yields and streamline production. The composition of substrates directly influences microgreen flavour and texture, necessitating significant advancements in this realm to enhance appeal to consumers and chefs alike.

This study delves into the realm of leguminous microgreens cultivation, focusing on advancing substrates and methodologies to optimize both nutritional content and production efficiency. By exploring innovative cultivation techniques and novel substrates, we aim to unlock the full potential of leguminous microgreens, enriching their flavour, texture, and most importantly, their nutritional value.

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2 Overview of Nutritional Benefits

The market for microgreens has expanded significantly, offering a plethora of options ranging from juices and teas to powders, pre-mixes, and tablets. These miniature versions of leafy greens pack a powerful nutritional punch, as evidenced by recent research findings. Various phenolic acids, including gallic acid, p-coumaric acid, and chlorogenic acid have been identified in chickpea and lentil microgreens (Ghumman et al. 2017). Building on this, Kurian and Megha (2020) discovered that chickpea microgreens boast higher vitamin content and antioxidant activity compared to their raw seeds or sprouts. Nutritional richness of lentil microgreens, particularly in polyamines like agmatine and spermidine, surpassing that of lentil sprouts was also highlighted (Kralj Cigić et al. 2020). Mung

beans, known for their balanced nutritional profile and widespread use in the food industry (Tang et al. 2014), have also been found to contain significant reserves of phenolics, flavonoids, minerals, and amino acids in their microgreen form (Kaur et al. 2022). These natural polyphenols found in pulse microgreens offer numerous health benefits, acting as scavengers of free radicals, inhibitors of radical species, and metal chelators to counteract oxidation processes.

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3 Importance of Leguminous Microgreens

Legumes are highly significant as human food crops, ranking second only to cereals (Nair et al. 2013). Grain legumes play a crucial role in meeting human dietary protein nitrogen (N) requirements, contributing approximately 33% of the needed protein nitrogen (Vance et al. 2000). In subsistence agriculture systems prevalent in tropical and subtropical regions, legume protein nitrogen in the human diet can exceed this figure, reaching twice the proportion found in more developed areas.

The levels of phytonutrients in plants exhibit variation across different growth stages. Research has indicated a decrease in phytonutrient content from the seedling (sprout/microgreen) to the fully grown stage in various plant species, including lettuce (Pinto et al. 2015), brassicas (Sun et al. 2013), broccoli (Guo et al. 2013), several types of beans (Nakamura et al. 2001), and amaranth (Ebert et al. 2015). Germination of seeds has been observed to enhance the nutritive value of legumes by activating enzymes that reduce or eliminate anti-nutritional factors (Bau et al. 1997). Additionally, germination has been found effective in reducing levels of phytic acid, stachyose, and raffinose in mungbeans while aiding in

better mineral retention compared to other processing methods such as dehulling, boiling, autoclaving, and microwave cooking (Mubarak 2005).

A diverse array of seeds has been utilized for cultivating microgreens, encompassing arugula, celery, red beet, green basil, and Tartary buckwheat, derived from families including Brassicaceae, Apiaceae, Amaranthaceae, Chenopodiaceae, Lamiaceae, and Poaceae, respectively. Conversely, certain plants such as tomatoes, peppers, eggplants, and others containing antinutrients during their seedling stages are unsuitable for microgreen cultivation. Notably, mature plants of legumes, grains, and sunflowers are inedible, their seeds offer notable nutritional benefits, rendering their microgreens edible (Table 1). For instance, certain legume microgreens exhibit lower levels of antinutrients such as phytic acid and tannins compared to their seeds. In the case of germinated peanuts, seed proteins undergo significant alterations reducing allergenic protein concentrations, making microgreens safer for individuals with peanut allergies (Du et al. 2022).

Table 1 Leguminous species analysed in studies investigating microgreens production

Species	Botanical name	Source
Mung bean	*Vigna radiata* L.	Kaur et al. (2022); Dhaka et al. (2023)
Lentil	*Lens culinaris* L.	Dhaka et al. (2023)
Pea	*Pisum sativum*	Li et al. (2023)
Chickpea	*Cicer arietinum*	Khalil et al. (2007); Kaur et al. (2022)
Black gram	*Vigna mungo*	Kaur et al. (2022)

4 Innovations in Cultivation Methods for Leguminous Microgreens

Owing to the reduction of agricultural land worldwide and the scarcity of freshwater resources, indoor vertical farming has become more and more popular. As a modern planting technology, hydroponics has been widely used in urban agricultural production.

4.1 Controlled Environmental Agriculture (CEA)

Microgreens represent a key component of the growing movement toward controlled environmental agriculture (CEA), particularly in indoor vegetable production (Riggio et al. 2019). Their unique characteristics make them adaptable to various environments, thriving year-round with minimal additional nutrient requirements. This versatility allows for cultivation in diverse spaces, including open areas, balconies, windowsills, and indoor spaces within homes, making them particularly promising for urban agriculture. Presently, large-scale microgreen cultivation predominantly occurs in greenhouses, effectively shielding crops from environmental pollution and ensuring year-round availability (Du et al. 2022). Various cultivation methods, encompassing outdoor, indoor, vertical, and greenhouse settings, employ controlled environmental conditions such as temperature, humidity, and lighting, including LED and fluorescent lamps. Nutrient solutions, foliar sprays, and growing mediums like hydroponics, inorganic substrates, and growing pads/mats are commonly utilized (Palmitessa et al. 2020; Petropoulos et al. 2021; Germ et al. 2019; Moraru et al. 2022). Despite their brief growing phase, commercial microgreen production demands meticulous attention, with the choice of a suitable growth medium significantly influencing yield, quality, and environmental sustainability (Di Gioia et al. 2016). Among commercial mediums, soil and cocopeat emerge as the most prominent and widely

accepted options, with cocopeat being identified as highly suitable for microgreen cultivation (Gunjal et al. 2024).

4.2 Hydroponic System

Hydroponics frequently makes use of nutrient-supplied recycled irrigation water to increase water resource utilization efficiency. To improve quality parameters, hydroponic microgreen production needs to be carefully planned and managed to control environmental factors (Rusu et al. 2021; Kyriacou et al. 2021; Šamec et al. 2022). Taking into account all the controllable aspects of hydroponic systems that have been demonstrated to affect the build-up of bioactive substances (Lam et al. 2020; Franzoni et al. 2022), the timing of harvest, and the quality of the final product (Birlanga et al. 2022; Liszka-Skoczylas et al. 2022), this is in contrast to more traditional production methods that employ soil. Moreover, in hydroponic systems, the absence of a soil microbiome is crucial to take into account because a poor parameterization process exposes the plants to detrimental microbial spoiling (Riggio et al. 2019; Nissen et al. 2021; Sathyanarayana et al. 2021).

4.3 Aeroponic System

Aeroponics represents the production of crops under air or mist environment without the use of soil as the rooting medium. The plant roots in this system are open to the environment and are free to spread out. This system has the benefit of using only 10% of the water required for hydroponics. It doesn’t seem like aquaponics growing is something that microgreen producers do very often. Hydroponic and aquaponic systems both have advantages in the production of microgreens. The system’s drawbacks include the possibility of crop death in the event of a malfunction that would halt the flow of nutrient solution in the form of mist (Savvas 2003).

4.4 Vertical Farming Techniques

Vertical farming facilitates the production of high value crops with higher yield than obtained from conventional farming by efficient utilization of resources such as water, nutrients, space and time, thereby, reducing carbon footprint (Specht et al. 2014). Vertical farming technology does not require huge arable land to produce crops and thus is agriculturally independent. This innovation utilizes both the horizontal and vertical spaces more effectively for microgreens, thereby, producing higher yield per unit volume under controlled environmental conditions of temperature, light, carbon dioxide and humidity.

4.5 Biofortification Techniques

Agronomic biofortification presents a practical and efficient method to enhance specific mineral levels in crops, contrasting with more complex breeding or genetic modification processes (Marques et al. 2021). Microgreens, with their dense nutrient content, rapid growth, and low levels of antinutrients like phytic acid, emerge as prime candidates for biofortification efforts (Di Gioia et al. 2019, 2021; Kyriacou et al. 2016; Poudel et al. 2023a). They respond well to nutrient inputs and can be grown in minimal space, offering high nutritional value and suitability for raw consumption (Di Gioia et al. 2017a, 2023; Poudel et al. 2023a, b).

Biofortification of microgreens with targeted minerals can be achieved through various methods such as seed nutri-priming, foliar spraying, and fertigation, with the latter being the most prevalent and effective technique (Di Gioia et al. 2019; Puccinelli et al. 2019; Poudel et al. 2023a, b). While successful applications of agronomic biofortification with zinc have been demonstrated in mature crops, detailed insights regarding optimal zinc sources and application rates for specific microgreen

species are still lacking (Barrameda-Medina et al. 2017a, b; D'Imperio et al. 2022; Ciriello et al. 2022; Velázquez-Gamboa et al. 2021; Di Gioia et al. 2019).

The biofortification process can significantly enhance the nutritional profile of microgreens, contributing to health-promoting diets and addressing nutrient deficiencies and chronic diseases (Choe et al. 2018). Moreover, it offers a more accessible pathway for nutrient enhancement compared to traditional growing systems, thereby advancing food and nutrition security (Di Gioia et al. 2019; Rouphael et al. 2018; Giordano et al. 2019).

Current efforts in biofortification primarily target on selected nutrients such as zinc (Zn), selenium (Se), and iron (Fe). For instance, Di Gioia et al. (2019) and Poudel et al. (2023a, b) achieved zinc biofortification of microgreens through nutrient solution enrichment and seed nutri-priming, respectively, resulting in significant increases in zinc content in Brassicaceae microgreens (Di Gioia et al. 2019) and sunflower and pea microgreens (Poudel et al. 2023a, b). Furthermore, several studies have explored the effects of selenium-enriched nutrient solutions on selenium content in microgreens (Islam et al. 2020; Mezeyová et al. 2022; Newman et al. 2021; Pannico et al. 2020; Puccinelli et al. 2019). Frąszczak and Kleiber (2022) investigated the impact of iron-supplemented nutrient solutions on iron content in hydroponically-grown purple kohlrabi, radish, pea, and spinach microgreens, revealing that specific nutrient concentrations positively influenced species-specific iron accumulation. Enhancing multiple nutrients simultaneously through biofortification provides a sustainable approach to combat various nutrient deficiencies and improve bioavailability for human consumption. In this research, arugula microgreens subjected to biofortification exhibited elevated levels of essential nutrients including vitamin C, zinc (Zn), calcium (Ca), sodium (Na), and potassium (K) (Kathi et al. 2024).

5 Enhancing Nutritional Quality Through Cultivation Substrate

5.1 Cultivation Substrate

Peat-based media rank as the most commonly employed substrate for microgreen production, with coconut coir and various synthetic media following closely behind. Though, they come with drawbacks such as high cost and non-renewability. An eco-friendly alternative to peat is coconut coir, which is organic and replenishable. However, coconut coir poses challenges due to potential salt accumulation and elevated levels of fungal and bacterial contaminants (Di Gioia et al. 2016; Pardossi et al. 2018). A cost-effective and sustainable substitute could involve utilizing fibrous materials like polyester, cotton, or jute fiber. Jute fiber mats offer several advantages as lightweight, biodegradable growth substrates, leaving no soil residues on the seedlings. Jute substrates could serve as a sustainable alternative for microgreen cultivation to peat, maintaining quality and yield while also reducing post-harvest costs (Min Allah et al. 2023). Inorganic options such as prlite, vermiculite, and rockwool are also available, but they tend to be pricey, energy-intensive to produce, and challenging to dispose of post-production (Bulgari et al. 2021). Utilizing locally sourced organic waste compost in combination with perlite mixture (50:50) has the potential to diminish the microgreens industry’s dependence on peat, concurrently cutting expenses and enhancing production sustainability. Blending organic waste compost with perlite mixture has the potential to yield microgreens with similar or superior nutritional quality when contrasted with perlite mixture alone (Poudel et al. 2023a).

Different natural and synthetic substrates (agave fiber, coconut fiber, peat moss, capillary mat, and cellulose sponge) affect the nutritional and phytochemical profiles of

microgreens in controlled conditions. Natural fiber substrates were reported to increase nitrate and macro-mineral levels compared to synthetic ones. Controlled stress on microgreens grown on peat moss could enhance phytochemicals without compromising growth. Nitrate deprivation may reduce consumer nitrate exposure in microgreens grown on natural fiber substrates (Kyriacou et al. 2020).

Leguminous microgreens like black gram, mung bean, and chickpea resulted altered levels of minerals, chlorophyll, aminoacids, phenolics and antioxidents when grown under different conditions like soil, water and cocopeat with nutrient solutions. Elevated levels of phenolic acids such as protocatechuic, p-coumaric, chlorogenic, caffeic, gallic, and ferulic acids, along with flavonoids including epicatechin, catechin, rutin, quercetin, kaempferol, vitexin, and iso vitexin, were found in soil grown microgreens. Microgreen extract powder had higher levels of amino acids when grown under coco-peat with nutrient solution. Soil-grown chickpea microgreens displayed the highest ash content, total chlorophyll content, mineral content (specifically magnesium, potassium, and calcium), total phenolic content, total flavonoid content, and total antioxidant activity among the microgreens examined. Conversely, microgreens of mung beans grown under coco-peat with nutrient solution demonstrated abundant amino acids in their microgreens extract powder (Kaur et al. 2022) (Fig. 1).

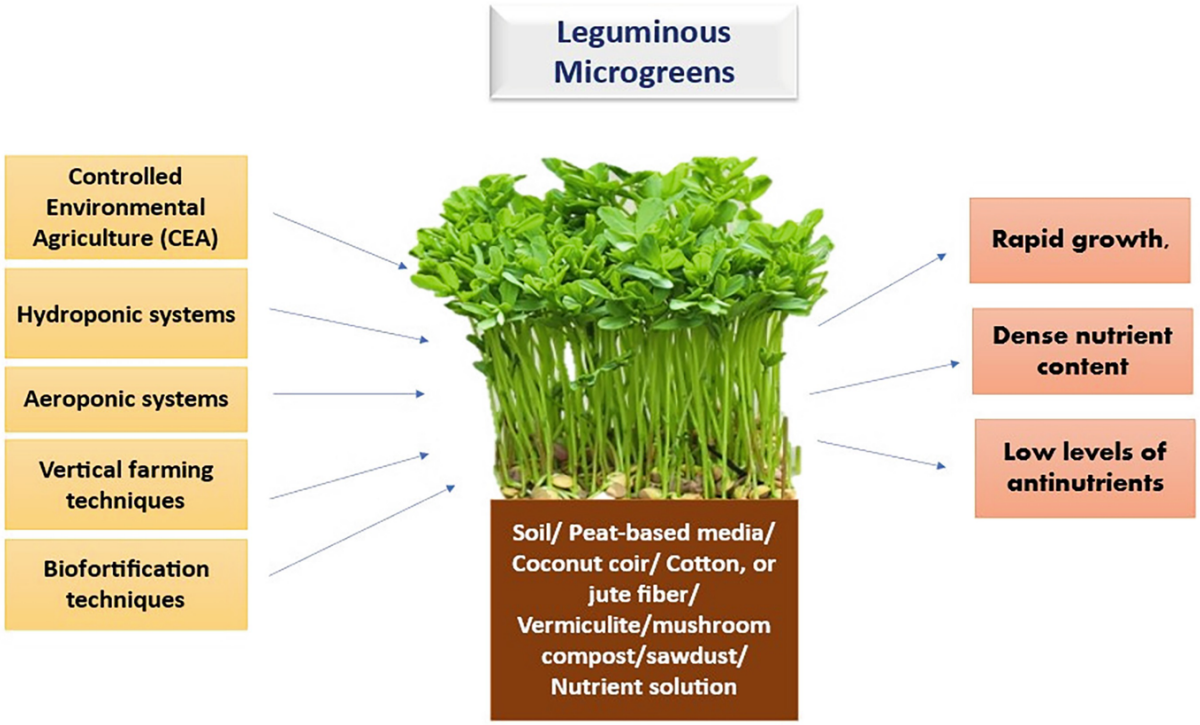


Fig. 1 Enhancing growth and nutritional quality of leguminous microgreens through cultivation substrates and techniques

A mixture of 30% vermicompost, 30% mushroom compost, 20% sawdust, and 20% perlite in potting medium was reported to increase yield of microgreen (Saleh et al. 2022). D'Imperio et al. (2021) obtained higher yields on 100% peat or a lower proportion of Posidonia (*Posidonia oceanica* L.) residues mix than if it was increased to 50% as a growing medium in the cultivation of Brassica microgreens. Different macro-micro-nutrients (total N, P, K, Ca, Mg, Fe, and S) were reported maximum in 75:25 compost: peat growing medium whereas maximum yield was for 50:50 compost: peat growing medium (Nájera et al. 2024).

The adoption of hydrogel-based soil less cultivation represents a novel approach, uniquely positioned to address both food safety and sustainability concerns, in cultivation of microgreens for urban agriculture. Over a 12-day growth period, the hydrogel consistently provided water to red cabbage microgreen plants, eliminating the

necessity for manual watering or supervision. Additionally, the incorporation of GMP (Sungro Metro-Mix® 360 growing mix particles; the primary constituents of the GMP include vermiculite, peat moss, tree bark, and dolomite lime.) enhanced nutrient levels and porosity, leading to a notable boost in microgreen yield (Teng et al. 2022).

5.2 Nutrient Supplementation Strategies

Microgreens cultivation, with unique requirements and early harvesting, demands nuanced nutrient management to prevent adverse outcomes. Abiotic stressors, such as water and nutrient deficiencies, significantly impact plant growth and quality, yielding potential positive or negative effects on crops (Schlering et al. 2020). Employing nutrient deprivation techniques is a cost-effective strategy, inducing the production of vital secondary metabolites for stress adaptation. This method allows precise regulation of nutrient solution composition to optimize plant growth and quality in hydroponic systems (El-Nakhel et al. 2019). While explored in field crops, it exploits plants' ionome plasticity and specific gene expression controlling root transporters (Courbet et al. 2021; Petropoulos et al. 2021).

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6 Conclusion

Numerous studies have explored the advancement of cultivation substrates and methods aimed at enhancing the nutritional quality and production of leguminous microgreens. Despite their short growing phase, commercial microgreen production demands meticulous attention, with the selection of a suitable growth medium and cultivation technique significantly impacting yield, quality, and environmental sustainability. Various cultivation methods, including outdoor, indoor, vertical, and greenhouse settings, utilize controlled environmental

conditions such as temperature, humidity, and lighting, including LED and fluorescent lamps. Additionally, nutrient solutions, foliar sprays, and growing mediums such as hydroponics, inorganic substrates, and growing pads/mats are commonly employed. Soil, cocopeat, compost stand out among commercial mediums as the most prominent and widely accepted options, with cocopeat particularly noted for its suitability in microgreen cultivation. From selecting appropriate substrates to implementing precise environmental conditions, each aspect of cultivation plays a crucial role in shaping the final product. Moreover, exploration of novel methods, such as Controlled Environmental Agriculture (CEA), hydroponic, aeroponic, vertical farming and biofortification techniques have revealed promising avenues for further innovation in microgreen production.

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Role of Mycorrhizal Association in Leguminous Plant Growth

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Keywords Fungi – Leguminous plant – Mycorrhiza – Plant growth – Symbiosis

Abbreviation

AM Arbuscular mycorrhiza

AMF Arbuscular mycorrhizal fungi

FAO Food and Agriculture Organization

N_2 Nitrogen

PGPR Plant growth promoting rhizobacteria

1 Introduction

One kind of non-rhizobia inoculant that legumes can respond to by altering plant development or the legume's own function is arbuscular mycorrhizal (AM) fungi (Chalk et al. 2006). Leguminous plants have a close interaction with arbuscular mycorrhizal fungus (AMF) and *Rhizobium* spp. Root nodules contain rhizobia, and the root cortex is colonised by AMF. Tripartite symbiotic relationships improve plant performance in a variety of environmental conditions, including growth, yield, and nutritional content. Arbuscular mycorrhizal fungi, which are found in a wide range of soil types globally, have symbiotic connections with 80% of all terrestrial plants. Benefiting the host, mycorrhiza mobilises phosphorus from non-labile sources (Soumare et al. 2015). Because it can enhance nutrient intake, the AM fungus is quite interesting. Apart from regulating the nutrient cycle in natural ecosystems, the activity of the AM fungus is crucial in determining the feasibility of managing this symbiosis in disturbed habitats. The increases in growth of the wild bean plants were consistent with the measurements of N_2-fixation activities and nodule development. Still, it seemed that the AM fungus's colonisation of the wild bean plants was more significant than the R-cell-produced nodulations (Eom et al. 1994).

The belief that Rhizobium and AM might compete with one another for colonisation sites on legume roots has been investigated, potentially reducing the efficacy of the symbiosis. Arbuscular mycorrhizal (AM) fungi are found in soil and naturally invade many different types of plant roots. AM has the capacity to provide the host plant with

inorganic nitrogen by directly absorbing it from the soil (Chalk et al. 2006). The “mutualistic” and “symbiotic” link between legume and rhizobium. Due to the mutualistic benefits shared by both, the relationship between the bacterium and the plant is symbiotic. There is host-specific interaction between rhizobia and legumes. This shows that not all rhizobia nodulate every legume, or that some species of rhizobia are specialised to modifying a given plant. Both broad and limited host range rhizobia are categorised based on how particular they are to their hosts. *Rhizobium leguminosarum* bvs. viciae and trifolii, for example, are distinct for their legume partner, despite their close relationship. Whereas *R. leguminosarum* bv. *trifolii nodulates*, *Vicia*, *Lathyrus*, *Lens*, *Pisum*, and *R. leguminosarum* bv. *viciae* do not (Soumare et al. 2015).

A large surface area is available for resource exchange in fungi that have undergone changes to form coils and arbuscules. Moreover, intraradical vesicles, which function as fungal storage devices, are produced by certain AM fungal species. Nuclei and other elements can move very easily inside the mycelium of AM fungus because its extraradical hyphae lack typical cross walls (Mohammadi et al. 2011). The Food and Agriculture Organisation of the United Nations (FAO) projects that by 2030, the world’s demand for agricultural products will have increased by 60% from its current levels, with emerging countries bearing the brunt of this rise at nearly 85%. The globe has been depending on rising agricultural yields for more than 50 years to keep up with the increasing demand for food. An agro-ecosystem’s sustainable output is largely determined by its soil (Abd-Alla et al. 2014). To boost the quantity and quality of commercially important legumes, farmers frequently utilise chemical fertilisers; however, using biofertilizers is a more cost-effective and environmentally responsible solution. Chemical fertilisers

have the potential to degrade soil quality by accumulating hazardous salt (Havugimana et al. 2016). Three herbs were planted next to *the R. pseudoacacia* legume tree: lucerne (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), and ryegrass (*Lolium perenn*e L.). The three species of herbal plants, which can be found in a range of settings all over the world, were considered to be the most important pasture and ground cover herbs (Yang et al. 2016).

Though the term “mycorrhiza” implies that fungi are related to roots, there are relationships known as mycorrhizal associations—which have to do with taking up nutrients from the soil—between hyphal fungi and the underground organs of many pteridophytes and bryophytes, as well as between seed plants’ roots and the sporophytes of most pteridophytes. Both ectomycorrhizas and endomycorrhizas are the two main types of mycorrhizas; they differ substantially in their structural composition and physiological relationships with symbionts (Mohammadi et al. 2011). Due of the multitude of uses for legumes, there is interest in their use worldwide. Since legumes (Fabaceae, Syn. Leguminosae) have intimate relationships with a variety of rhizosphere microorganisms, they are also essential components of terrestrial ecosystems that are found naturally as well as those that are artificially produced. Arbuscular mycorrhizal fungi (AMF) are a universally distributed kind of soil microbiota. They work in symbiotic partnerships with the roots of many different kinds of plants to perform roles such as biofertilizers, bioprotectants, myco-remediators, and bio-degraders. Applying AMF alone has been found to improve the overall performance of leguminous plants grown using a variety of agricultural practices. Furthermore, the interaction of the AM fungus with other plant-growth-promoting rhizobacteria has been observed to significantly enhance the development and yield of legumes (Muleta

2017). In addition to providing food for people and animals, legumes are useful for enhancing soil in agroforestry and agricultural systems when mixed with biofertilizers. In order to promote healthy plant growth, the latter may contain ingredients that are good to the environment. Solutions containing the cells of live microorganisms are known as bio-fertilizers, and they help agricultural plants absorb nutrients by interacting with the rhizosphere. Arbuscular mycorrhizal (AM) symbionts are fungi that are beneficial to plant growth. To boost the quantity and quality of commercially important legumes, farmers frequently utilise chemical fertilisers; however, using biofertilizers is a more cost-effective and environmentally responsible solution. The use of chemical fertilisers on soil can have negative consequences (Havugimana et al. 2016).

Studies measuring the contribution of arbuscular mycorrhiza (AM) to the legume–Rhizobium symbiosis are scarce. However, the advantages that native and inoculated AM can provide for legume development, nitrogen uptake, and atmospheric N_2 proportionality have not been conclusively shown in many research. The more widely recognized advantage is higher dry matter content in the legumes or higher grain yield; yet, in some circumstances, AM can enhance Path as well as legume growth (Chalk et al. 2006). Legumes' primary feature is their ability to fix nitrogen with the help of a bacterium that lives in their root nodules. Currently, there are known to be 13,000 species of legumes over 700 taxa and 98 species of bacteria that nodulate legumes. They have a major role in the global nitrogen flux, which makes them essential for both ecological and agriculture. They also provide food for humans and animals by acting as a source of protein. In order to improve the quality of the soil and increase its organic matter content, legumes are often used as green manure in agriculture (Zahran 1999; Soumare et al. 2015).

The fungi in question are members of the monophyletic phylum Glomeromycota, which comprises approximately 160 species. Arbuscular mycorrhizas are sometimes referred to as “endomycorrhizas” since the fungus partner forms intraradical structures inside plant roots. In AM partnerships, arbuscules or coils act as a mediator between the fungal and plant tissues, allowing materials to be exchanged between the two symbionts (Mohammadi et al. 2011). Biofertilizers contain a variety of microorganisms that can alter the availability of essential nutrients in the soil through biological processes. Around 65% of the nitrogen required by crops worldwide is supplied by microbial inoculants and biofertilizers, which are extensively used in organic farming. Comparing microbial inoculants for biofertilizers to traditional or synthetic pesticides and fertilisers, there are several advantages (Pierre et al. 2014). Arbuscular mycorrhizal fungi (AMF), a type of soil microbe, cohabit peacefully with 80–90% of vascular plant species and 90% of agricultural plants. This group includes most agricultural crops, including those used for food, vegetables, and horticulture (Smith and Read 2010). They are extensively dispersed across the world’s ecosystems, which are mostly governed by the variety of acknowledged plant hosts (Diagne et al. 2020).

AMF comprise around 250 species, 25 genera, and 11 families. Arbuscular mycorrhizal (AM) symbiosis is arguably the most common beneficial plant-microorganism relationship. Actinorhizal plants perform better in a variety of settings due to the combined benefits of AMF and the nitrogen-fixing actinobacteria Frankia, as was seen in legumes (Yang et al. 2016). Additionally acknowledged are the benefits of the tripartite symbiosis of ectomycorrhizal fungus, nitrogen-fixing bacteria, PGPR, and AMF. The combined height and biomass of *C. equisetifolia* plants increased significantly upon inoculation with a blend of

AMF, *Frankia*, *Azospirillum*, and *Phosphobacterium*. The same scientists noticed enhanced nutrient uptake of N, P, K, Ca, and Mg in triple-inoculated plants (Parniske 2008).

Numerous studies have provided evidence in favour of the theory that AM helps legumes by increasing their uptake of P, particularly during seedling growth. Even in unfertilized soil, positive reactions to AM inoculation can happen; although, they are more prevalent in soils that have received superphosphate treatment to address low available P status (Chalk et al. 2006). There are six billion people on the earth as of right now. It is anticipated that by 2040, there will be eight or nine billion people on the planet. The main difficulty will be to maintain a consistent supply of food to feed this expanding population (Soumare et al. 2015). The majority of people think that roots and AM fungus have a mutualistic, symbiotic connection. Identifying the ideal plant-fungus combination for each distinct ecological and environmental situation is one of the main challenges facing AM research at the moment. Therefore, one needs to be aware of the several factors governing the population biology of AM fungi in order to use them in sustainable agriculture. Mycorrhizae are beneficial soil fungi that coexist with plant roots in symbiotic interactions. They have a critical role in increasing the uptake of Zn and Cu by plants, as well as the uptake of P and S and other weakly mobile micronutrients (Molla and Solaiman 2009). A little over half of all nitrogen fixed by biological means comes via symbiotic nitrogen fixation. *Rhizobia and Frankia* are two symbiotic groups that fix nitrogen from the atmosphere. Of them, the symbiosis between rhizobia and legumes accounts for about 45% of the nitrogen used in agriculture. Rhizobia and legumes are diverse (Soumare et al. 2015). Ectocorrhizas and endomycorrhizas are the two primary forms of mycorrhizas; they differ in their physiological interactions

and structural features with their host plant. Mycorrhizal fungi spread far beyond root surfaces and thrive in soil pores that are too tiny for root hairs to penetrate. As a result, they frequently encourage plants to reduce the biomass of their roots while also improving their capacity to absorb nutrients. Fungi that penetrate and colonise legume roots are both mycorrhizal and rhizobial. Arbuscular mycorrhizal (AM) fungi affect the surrounding soil biota, the eco-physiology, and related non-legumes of legumes in a fundamental way (Mohammadi et al. 2011).

It is impossible to overestimate the significance of using traditional chemical fertilisers to improve soil quality, fertility, biology, and agricultural productivity as a result of improved crop yield. However, because the cost of fertilisers is rising and their impacts on the chemical characteristics and health of plants and soil typically correlate with rising energy prices, these must be complemented or replaced with less expensive options such organic amendments, biofertilizers, and beneficial microbial inoculants (Pierre et al. 2014).

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2 Diversity of Arbuscular Mycorrhiza

Fungi is the most diversified group of thalophytic organism and representing in separate Kingdome Fungi (Singh and Singh 2022). Fungi revelling in all type of associated forms of life as saprotrophic, parasitic and symbiotic (Singh and Singh 2023). Mycorrhiza is one of the important and beneficial mutualistic association of fungi with higher plants. Arbuscular mycorrhizal fungi (AMF) are obligate symbionts that require a terrestrial host plant (Paredes-Jácome et al. 2022). Plant development and growth in terrestrial habitats depend on Arbuscular Mycorrhizal Fungi (AMF) to strengthen plants against abiotic stressors. Arbuscular Mycorrhizal Fungi (AMF) develop alongside higher plants' roots, particularly those of leguminous

plants, to help them absorb nutrients and minerals. This improves plant growth and productivity, even in the face of stress. Carbohydrates are given to these AMF in exchange for completing their life cycle. AMFs function as a type of biofertilizer that improves soil health and ecosystem stability, hence fortifying crop plant quality (Singh et al. 2022). In the AMF and terrestrial host plants, both partners benefiting from reciprocal nutrient exchange (Dobo 2022). Many different types of terrestrial plants have their roots colonized by the mycelium of AMF, forming a common mycorrhizal network. Many plant populations are impacted by the common mycorrhizal network, which is an essential part of the terrestrial ecosystem (Pringle et al. 2009). A verities of host plants with the association of AMF's, described, many species including relevant to the crops (Diagne et al. 2020) such as *Solanum lycopersicum* (Gamalero et al. 2003; Bona et al. 2016), *Sorghum bicolor* (Kim et al. 2017), *Withania somnifera* (Parihar and Bora 2018), *Cucurbita maxima* (Al-Hmoud and Al-Momany 2017), *Piper longum* (Gogoi 2011), *Phaseolus vulgaris* (Ibijbijen et al. 1996), *Panicum hemitomon* (Miller and Sharitz 2000), *Citrullus lanatus* (Ban et al. 2011), *Musa acuminata* (Rodríguez-Romero et al. 2005), and *Prunus cerasifera* (Berta et al. 1995) etc. Approximate 2000 species of fungi known for the mycorrhizal relationship with higher plants. These species are forming mycorrhizal symbiotic relationship in two types viz. Ectomycorrhiza and Arbuscular Mycorrhiza (Ianson and Smeenk 2010). Diversity of AMF in higher plants are listed in Table 1.

Table 1 Diversity of AMFs in higher plants and their responses

AMF's	Host Plant	Responses	References
Acaulospora trappei, *Scutellospora heterogama*,	*Panicum hemitomon*, *Leersia hexandra*	Improve phosphorus (P) nutrition	Miller and Sharitz (2000)

AMF's	Host Plant	Responses	References
Glomus leptotichum, Glomus gerdemannii			
C. etunicatum	*Cucumis sativus*	Increasing antioxidant enzyme activity, biomass, and pigment production	Hashem et al. (2018)
C. etunicatum, R. irregularis, F. mosseae	*Glycine max*	Safeguarded soybean genotypes against membrane damage caused by salt	Hashem et al. (2016)
F. mosseae	*Elymus nutans*	Accelerated plant growth and raised chlorophyll levels	Chu et al. (2016)
F. mosseae	*Lycopersicon esculentum*	Accelerated fruit weight and yield growth	Latef and Chaoxing (2011)
F. mosseae	*Gossypium arboreum*	Increased biomass and phophorus concentrations	Tian et al. (2004)
F. mosseae	*Trigonella foenum-graceum*	Better plant performance	Kelkar and Bhalerao (2013)
F. mosseae, R. irregularis	*Populus alba, Populus nigra*	Alleviation of Cu and Zn phytotoxicity	Lingua et al. (2008)
Funneliformis mosseae	*Solanum lycopersicum*	Increased plant height and biomass and stomatal density	Chitarra et al. (2016)
Funneliformis mosseae, Paraglomus occultum	*Poncirus trifoliata*	Enhanced hyphal length, hyphal water absorption rate, and leaf water potential	Begum et al. (2019)
Funneliformis sps.	*Zea mays*	Regulate photosystem (PS)- II heterogeneity	Mathur and Jajoo (2020)
G. claroideum	*Triticum aestivum*	Increased leaf chlorophyll concentration and overall dry weight	Khan et al. (2022)

AMF's	Host Plant	Responses	References
G. clarum, *Acaulospora scrobiculata*, *Gigaspora rosea*	*Phaseolus vulgaris*	Increased root dry matter and root length density	Hu et al. (2022)
G. deserticola, *G. gigantea*	*Vigna unguiculata*	Increased mineral elements uptake and improve water relations; to improve stomatal control, increase the hydraulic conductivity of the roots	Hu et al. (2022)
G. etunicatum, *F. mosseae*, *R. irregularis*	*Triticum aestivum*	Increased plant growth, nutrient uptake and grain yield	Daei et al. (2009)
G. fasciculatum, *G. mosseae*, *G.aggregatum*	*Vigna unguiculata*	Enhanced nutrient uptake, attributed to growth, plant height, number of nodules and productivity	Yaseen et al. (2011)
G. fasciculatum, *G. mosseae*, *G. intraradices*, *G. etunicatum*, *G. caledonium*	*Glycine max*, *Arachis hypogaea*, *Helianthus annuus*, *Carthamus tinctorius*, *Ricinus communis*	Increased the grain yield (production)	Solaiman et al. (2014)
G. versiforme, *R. irregularis*	*Hordeum vulgare*	Increasing the survival rate and low temperature stress	Hajiboland et al. (2019)
G. versiforme, *R. intraradices*	*Lonicera japonica*	Decreased Cd concentrations in shoots and roots	Jiang et al. (2016)
Glomus etunicatus	*Pistacia vera*	Increased shoot and root weights, leaf area and total chlorophyll	Abbaspour et al. (2012)
Glomus etunicatus	*Allium cepa*	Improved dry and fresh weights	Nelsen and Safir (1982)
Glomus geosporum	*Aster tripolium*	Manage higher concentrations of	Solís-Rodríguez et

AMF's	Host Plant	Responses	References
		soluble sugars	al. (2020)
Glomus intraradices	*Pterocarpus offcinalis*	Improve plant growth	Fougnies et al. (2006)
Glomus isolates	*Zea mays*	Enhanced essential elements (K, P, and Mg) and dry weight	Kaldorf et al. (1999)
Glomus mosseae	*Trifolium pratense*	Reductions in Zn absorption and concentrations in roots and shoots	Li and Christie (2001)
R. fasciculatum	*Cyclamen persicum*	Increased biomass generation and reaction to heat stress	Maya and Matsubara (2013)
R. fasciculatum	*Acacia nilotica*	Improved root and shoot biomass, and nutrient concentrations	Giri et al. (2007)
R. fasciculatus	*Triticum aestivum*	Enhanced stomatal conductance	Allen and Boosalis (1983)
R. fasciculatus, *Glomus macrocarpum*	*Acacia auriculiformis*	Increased root and shoot weights, and greater nutrient acquisition, changes in root morphology	Giri et al. (2003)
R. irregularis	*Lactuca sativa*	Improved shoot dry weight and stomatal conductance	Ruiz-Lozano et al. (2015)
R. irregularis	*Lycopersicon esculentum*	Improved leaf area, dry mass, and stomatal conductance	Dell'Amico et al. (2002)
R. irregularis	*Cucumis sativus*	Increases the photosynthetic efficiency of cold-stressed and seedlings	Ma et al. (2019)
R. irregularis	*Capsicum annuum*	Increased leaf area, mineral content, proline, sugars, and cell membrane integrity	Beltrano et al. (2013)

AMF's	Host Plant	Responses	References
R. irregularis	*Solanum lycopersicum*	Improved nutrient uptake, chlorophyll and proline concentrations, and dry weights of the shoots and roots	Hajiboland et al. (2009)
R. irregularis	*Digitaria eriantha*	Increased stomatal conductance	Pedranzani et al. (2015)
Rhizophagus fasciculatus, *Ambispora leptoticha*	*Glycine max*	Increased the total length area of the plant and thus the photosynthetic activity and in turn; increased the yield production	Hu et al. (2022)
Rhizophagus irregularis	*Zea mays*, *Solanum lycopersicum*	Enhanced apoplastic water flow	Bárzana et al. (2012)
Rhizophagus irregularis, *Rhizophagus fasciculatus*	*L. esculatum*, *Capsicum annuum*	Improved biomass, root & shoot length	Padmavathi et al. (2016)
Rhizophagus irregularis	*Trifolium repens* *Lavandula spica*	Increased dry weight, nutrients (P, K, Ca, Mg, Zn and B) and relative water content	Ortiz et al. (2015)
Rhizophagus irregularis	*Cajanus cajan*	Enhanced proline production, nutritional status (P, N, Mg, and Fe), and root biomass	Begum et al. (2019)

3 Mycorrhizal Association with Leguminous Plants

Most terrestrial plants' roots associate symbiotically with fungus. These ubiquitous symbioses, referred to as mycorrhizas, serve as pathways for the transportation of nutrients and energy between soil and plants. Although the term "mycorrhiza" implies a relationship between fungi and roots, there are relationships known as "mycorrhizal

associations” among hyphal fungi as well as the subterranean structures of the gametophytes of numerous bryophytes along with pteridophytes, as well as between the roots of seed-bearing plants as well as the sporophytes of most pteridophytes. Such associations have an impact in the assimilation of nutrients from soil. Ecto and endomycorrhizas are the two primary forms of mycorrhizas, and they differ greatly with regard to of their physiological interactions with symbionts and their structural makeup (Cardon and Whitbeck 2007).

According to (Scheublin and Vander Heijden 2006), mycorrhiza helps the host by mobilising phosphorus from non-labile sources, whereas rhizobia fix nitrogen and enriches the soil and plants with nitrogen (Hindumathi and Reddy 2012). Approximate 175 × 106 tonnes of nitrogen are estimated to have been collected globally each year in this manner. This helps to promote sustainable agriculture along with agroforestry by reducing reliance on chemical fertilisers. In addition to providing beneficial nutrition and having positive effects on soil aggregation, hydric condition, and mineral enrichment, amyloid fungi and rhizobia serve as biofertilizers and can, through biological mechanisms, reduce the frequency and severity of root diseases. (Gao et al. 2012; Hindumathi and Reddy 2012). Phytoremediation technology has also effectively employed them to eliminate heavy metals from soils. For development and survival, many plant species—especially legumes—rely entirely on microbial symbionts (Van Der Heijden et al. 2008).

Root nodule bacteria aids legumes in fixing nitrogen, which is their main characteristic. As of right now, 13 bacterial genera comprise 98 species of legume-nodulating bacteria, and 700 genera comprise 13,000 species of legumes (Rajwar et al. 2013). Because they account for a sizable portion of the worldwide nitrogen flux, they are crucial to agriculture and ecology alike. Additionally, they

offer a source of protein for animal and human nutrition. Legumes have a high organic matter content and are frequently utilised as green manure in agriculture (Zahran 1999). However, a number of environmental conditions, particularly in arid as well as semi-arid regions with frequent salt stress, droughts, low mineral availability, infections, and contaminated soils, inhibit the growth of legumes and result in significant yield losses. Consequently, abiotic stressors and nutrient deficiencies (Abdel-Fattah et al. 2010) must also be overcome by legumes produced in these places in addition to disease threat. Studies have demonstrated that synergistic interactions between AMF and rhizobia aid in the resolution of these issues. According to (Caetano-Anollés and Gresshoff 1991), the process of establishing a symbiotic link is both advantageous and expensive. However, plants have evolved autoregulation to reduce the costs associated with this process (Table 2).

Table 2 Reduced mineral stress in bean plants through rhizobia and AM inoculants

Legumes with associated mycorrhiza	**Mechanisms**	**Observation**	**References**
Trifoliate Orange (*Poncirus trifoliate*) with *Glomus mosseae* and *G. versiforme*	K^+, Mg^{2+} and Ca^2	Reduce the deleterious effects of salt stress	Zou and Wu (2011)
Vigna radiata cv. with *Bradyrhizobium* CAN-11 and *G. mosseae* BAS-I	Proline accumulation	Growth advancement	Diouf et al. (2005)
Vicia faba with *Glomus clarum*	K^+, Mg^{2+} and Ca^2	Growth advancement	Rabie and Almadini (2005)
Glomus fasciculatum/Rhizobium Cajanus cajan	Increased chlorophyll, nitrogen and phosphorus contents	Growth advancement	Bhattacharjee and Sharma (2012)

4 Role of AMF's

Intricate communication processes between the fungus and the plant are mediated by arbuscular mycorrhizal fungi (AMF), which lead to improved photosynthetic rate, greater water uptake, and other characteristics related to gas exchange (Birhane et al. 2012). This allows host plants to grow vigorously under stressful conditions. According to a number of studies, fungal symbiosis boosts resistance to a variety of stresses, such as salt, heat, drought, herbivory, metals, and illnesses (Ahanger et al. 2014, Rodriguez and Redman 2008; Soumare et al. 2015). Ahanger et al. (2014) found that about 90% of plant species, including ferns, bryophytes, and flowering plants, may form interdependent relationships with AMF. Arbuscular mycorrhizal fungi also create vesicles, arbuscules, and hyphae in the roots, but they also produce spores and hyphae in the rhizosphere. When the AMF creates a hyphal network with plant roots, it enhances plant development by providing roots with increased access to a vast amount of soil surface area (Bowles et al. 2016). According to Rouphael et al. (2015), AMF enhance plant nutrition by improving the availability and transport of several nutrients.

Arbuscular mycorrhizal fungi enhanced the quality of soil by inducing their structure and texture which increase plant health (Zou et al. 2016; Thirkell et al. 2017). The breakdown of organic materials in soil can be accelerated by fungus hyphae associated with plant roots (Paterson et al. 2016). Furthermore, by enhancing the "sink effect" and the transfer of photo-assimilates from the aerial portions to the roots, mycorrhizal fungi may influence the way in which host plants fix atmospheric CO_2. In light of the importance of AMF and the developments in research pertaining to their applications in agriculture, the current review focuses on the role of AMF as bio-fertilizers in the regulation of

plant growth and development with improved nutrient uptake under stressful environments, as well as the degree to which AMF can enhance plant growth under stressful environments. The role of AMF is to improve yields of plants through overall physio-chemical and biological functions (Fig. 1).

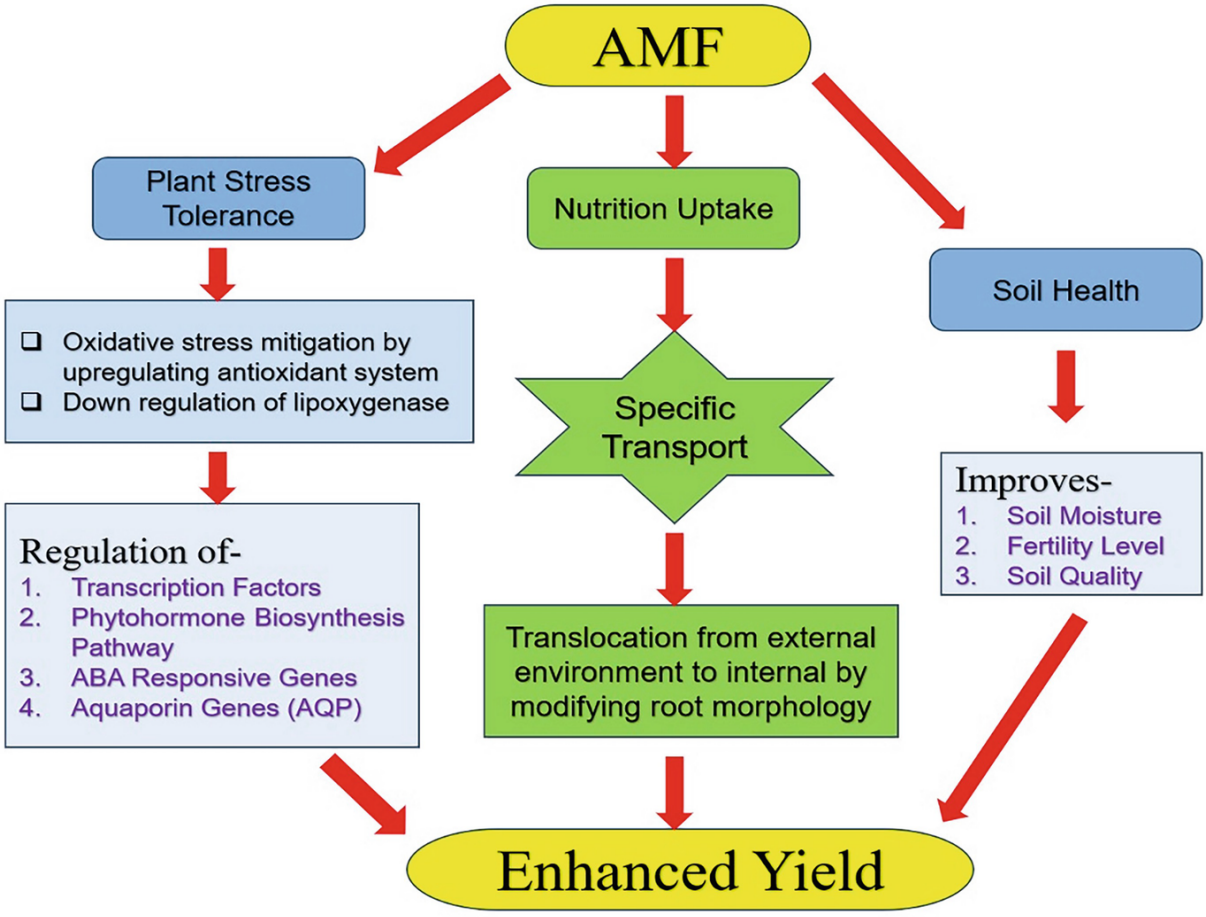


Fig. 1 Role of AMF in the enhancement of crop production (plant yields)

4.1 Arbuscular Mycorrhiza as Biofertilizer

The majority of terrestrial plants have symbiotic relationships with arbuscular mycorrhizal (AM) fungi, which are essential to the cycling of nutrients and the development of plants. This fungus, which are members of the phylum Glomeromycota, are particularly significant for improving the absorption of nutrients by plants, notably

phosphorus. It is crucial to comprehend the mutually beneficial effects of arbuscular mycorrhiza and plants on both parties when evaluating this symbiotic connection as a biofertilizer. Arbuscular mycorrhizal fungi, often known as AMF or AM fungi, are an important component of the soil microbial ecosystem that facilitate plant-soil nutrient uptake. As a result, their popularity as natural fertilizers are rising. According to Schubler et al. (2001), AMF are obligatory symbionts that are members of the phylum Glomeromycota, which together with over 80% of land plant species, develop mutualistic symbioses, with numerous agricultural crops as natural biofertilizers, namely Berruti et al. AM Fungi. In return for photosynthetic products, they supply water and mineral nutrients to the host plants (Smith and Read 2010). Nutrients from soil volumes inaccessible to roots can be obtained by the AMF mycelium that develops from the root system (Smith et al. 2003).

Depending on the kind of soil, applying farmyard manure might raise the densities of AM fungus spores (Harinikumar and Bagyaraj 1989). According to Kahiluoto et al. (2001), a number of studies have shown that cumulative P fertilisation reduces spore density in Northern European field settings. According to another research (Sylvia and Neal 1990), P supplementation inhibited root colonization when N was adequate, but had no effect on AM fungal colonisation when plants were lacking in N. Thus, the farmer may control the AM fungus at the agricultural site by using agronomic soil management techniques. In addition to crop selection, another crucial aspect of traditional agriculture is the use of fungicides, especially systemic fungicides, which have been demonstrated to inhibit AM fungus activity when used in the field (Menge et al. 1978; Kling and Jakobsen 1997). Certain fungicides may not always attack AM fungus. Based on tests conducted on groundnuts, mancozeb, thiram, and

ziram are dithiocarbamates that collectively seem to be harmful to mycorrhizal fungi (Sugavanam et al. 1994).

4.2 Arbuscular Mycorrhiza in Mineral Nutrition Enhancement

In order to help many plants survive and thrive under rather severe mineral stress circumstances arbuscular mycorrhizal fungi typically create beneficial and useful symbioses with their roots (Sieverding and Harris 1991; Sylvia and Williams 1992; Bethlenfalvay 1992). The capacity of host plants to resist or limit acquisition of minerals hazardous to growth (Marschner 1991) and improved host plant acquisition of mineral nutrients (Marschner and Dell 1994; Persad-Chinnery and Chinnery 1996) are some of the main advantages of mycorrhizae. The increased effective root surface areas that fungal hyphae give to enable more soil exploration and to get across mineral nutrient and water depletion zones close to active root surfaces may help to explain some of the beneficial benefits of root symbiosis with AMF. Compared to nonmycorrhizal (non-AM) plants, mycorrhizal (AM) plants frequently have greater diameters, more weight, length, and number in their roots (Hetrick et al. 1988).

4.3 Arbuscular Mycorrhiza Enhancement Inoculation

As members of the monophyletic phylum Glomeromycota, which is present in practically all terrestrial ecosystems, among the most widely spread endotrophic mycorrhizal fungal species are arbuscular mycorrhizal fungi (AMF) (Wu et al. 2016). With the exception of select Brassicaceae plants and species from a few other families, almost 90% of flowering plants, ferns, and bryophytes may develop a symbiotic interaction with AMF (Zhu et al. 2010; Liu et al. 2014). The hyphae, mycorrhiza, and vacuole in the root and

the hyphae and spore in the soil are the main constituents of arbuscular mycorrhizal fungi. Plants can create a vast hyphal network in their rhizosphere that improves the physical and chemical characteristics of soil, as well as plant development, yield, and crop quality (Rozpądek et al. 2016; Bowles et al. 2016), and facilitates the soil nutrients uptake (Chatzistathis et al. 2013; Baum 2015). AMF improves the nutritional state of plants by absorbing and moving mineral nutrients outside of the rhizospheric zone (Rouphael et al. 2015). Arbuscular mycorrhizae can readily form arbuscular mycorrhizal associations among vegetable crops, with the exception of Cruciferae, Chenopodiaceae, and Amaranthaceae, which are either incapable of being colonized by them or are less likely to do so. Other families that are more likely to experience this phenomenon include Leguminosae, Solanaceae, and Cucurbitaceae (Elbon and Whalen 2015).

Numerous plant species, including kidney beans, peppers, watermelons, muskmelon, onions, tomatoes, and asparagus, have been shown to benefit from AMF induced favorable effects on physiology and growth (Beltrano et al. 2013; Rozpądek et al. 2016). Additionally, we discovered that compared to non-AMF control plants, tomato seedlings infected with *Funneliformis mosseae* display considerably greater biomass, redox poise, and calcium balance (Liu et al. 2016). Legume crops, such as soybeans, can develop faster and have higher yields and nutritional value if AMF and rhizobia are carefully chosen and complementary to the host plant (Meghvansi et al. 2008).

5 Conclusion

Numerous studies have already confirmed the advantageous function of AMF in enhancing plant development and growth. Consequently, the material that

has already been available regarding the function of AMF has been compiled in this chapter in a logical manner to help readers comprehend the symbiotic relationship that AMF has with plants, particularly vegetables. Prior to now, AMF have primarily been talked about as helpful substances for soil mineral and nutrient uptake; however, it has now been demonstrated that plants injected with AMF can successfully fight a variety of environmental problems, including salinity, drought, alkalinity, nutrient stress, cold stress, and temperature stress. As a result, AMF contributes to the growth of several leguminous crops' plant yields. The application of AMF increases enormous crop yields and highlights the significance of the current global agricultural system for its continuous viability. In comparison to their symbiotic plant host, AMF diversity is far higher, demonstrating natural balance and improved plant care. AMF needs to be investigated at various other levels, like biofertilizer, to support sustainable agricultural production. By drastically lowering the usage of chemical fertilizers and other chemicals (inorganic, or synthetic), AMF for agricultural enhancement can reduce biological amplification and advance bio-healthy agriculture. To meet the growing demands of the global population, crop plants may benefit from the use of AMF-mediated yield and productivity enhancement.

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Microbial Consortia Unleashed: A New Era in Sustainable Microgreen Cultivation

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Keywords Cultivation – Microgreen – Microbial consortia – Nutrient uptake – Plant-microbe interaction

Abbreviations

AMF Arbuscular Mycorrhizal Fungi

CFU Colony-Forming Units

IAA Indole-3-Acetic Acid

Ca Calcium

Mg Magnesium

Na Sodium

Fe Iron

Mn Manganese

Zn Zinc

Se Selenium

Mo Molybdenum

cm Centimeter

K Potassium

N Nitrogen

P Phosphorus

S Sulfur

DW Dry Weight

FW Fresh Weight

PGPB Plant Growth Promoting Bacteria

AHL Acyl Homoserine Lactone

Da Dalton

VOCs Volatile Organic Compounds

ISR Induced Systemic Resistance

SAR Systemic Acquired Resistance

PGPMs Plant Growth-Promoting Microorganisms

NFT Nutrient Film Technology

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1 Introduction

Agricultural market trends have changed significantly over the last decade because of new cropping technologies adapted to people's demand for food diversification and supply, the impact of biotic and abiotic constraints, and climate changes that impact agricultural products (Paraschivu et al. 2021). With more than 10 billion people

expected by 2050, feeding the global population will become one of the most difficult issues for both scientists and farmers. Therefore, food security policies and efforts are mostly focused on assuring food quantity and everyday calorie consumption rather than enhancing food quality (Guardiola-Márquez et al. 2022). Current farming practices i.e. excess use of pesticides, land, high-yield varieties, crop intensification, and poor micronutrient fertilization are resulting in nutrient-deficient foods and also seriously affecting public health, degrading soil, and harming agroecosystems (Rabadán-Chávez et al. 2023; Acharya and Pal 2020). These current farming practices are connected with the micronutrient deficiencies in the population including iron and zinc, which are the most common micronutrient deficiencies around the world (Guardiola-Márquez et al. 2022). Furthermore, increased use of low-nutrient, low-cost, energy-dense, and processed meals has considerably contributed to the current worrying rise in nutritional deficiency and other long-term illnesses (Praharaj et al. 2021; Kathi et al. 2023). Therefore, there is an urgent need to develop more sustainable, inexpensive, accessible, and nutritious food products. In this regard, the utilization of microgreens has grown in favour in recent years.

Microgreens are rich in vitamins, minerals (Ca, Mg, Fe, Mn, Zn, Se and Mo), antioxidants and phytonutrients (ascorbic acid, β-carotene, α-tocopherol and phylloquinone). Microgreens are also becoming more prevalent in health-promoting diets, as they are a good source of nutritional and bioactive components that help avoid malnutrition and chronic diseases (Renna et al. 2020). Microgreens are plant seedlings, which are harvested after development of cotyledonary leaves, approximately 7–14 days post-germination (Ma et al. 2022; Zhang et al. 2021). These versatile crops can be cultivated with ease, speed, and affordability. Their eco-friendly

nature, stemming from minimal resource and equipment needs, makes them a vital option for dietary supplementation (Galieni et al. 2020). Additionally, microgreens have lower amounts of antinutrient and higher amounts of nutrients and bioactive compounds like amino acids, sugars, mineral, phenolic compounds, carotenoids and antioxidants as compared to mature plants (Tallei et al. 2024).

Interestingly, environmental stresses, both biotic and abiotic factors affect the production of these nutrients in microgreen. Therefore, several microbial consortia have been used to counterattack the effect of these stresses on microgreen production quality, and quantity. In this chapter, we highlight the effect of microbial consortium on microgreen cultivation as well as its application methods. Additionally, the chapter discusses the role of microbial consortia in enhancing the nutrient quality of microgreens and sustainable agriculture practices.

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2 What Are Microgreens and Their Use?

Microgreens are also known as sprouts, baby green that are used in salad mixtures. They are seedlings of edible plants, which were harvested 7–14 days after germination during cotyledonary leave's stage. In other terms, microgreens can be defined as germinated seedlings with fully developed and non-senescent cotyledons and the emergence and early development of one to two true leaves. They are typically harvested without roots. Basically, microgreen, baby green and sprouts are those green vegetables, which are harvested and consumed at immature stages. They can be differentiated on the basis of their age and size, sprouts are youngest less than 5 cm, while microgreens are older 5 cm long and baby green are

the oldest and 7.5 to 10 cm in length (Treadwell et al. 2020). Several varieties of generally grown vegetables were used to cultivate the microgreen due to their high rise and demand. These vegetables are belonged to several families such as Amaranthaceae, Amaryllidaceae, Apiaceae, Asteraceae, Boraginaceae, Brassicaceae, Convolvulaceae, Cucurbitaceae, Poaceae, Lamiaceae, Leguminosae, Onagraceae, Polygonaceae. The detail of some plants used as microgreens production available in Table 1.

Table 1 Some plants used for microgreen production

Common name	Species	Family
Cilantro, coriander	*Coriandrum sativum*	Apiaceae
Carrot	*Daucus carota*	
Parsley	*Petroselinum crispum*	
Table beet, Bull's blood beet	*Beta vulgaris* subsp. *vulgaris*	Amaranthaceae
Chard	*Beta vulgaris* subsp. *vulgaris*	
Amaranth	*Cicla group*, *Amaranthus* spp.	
Chives	*Allium schoenoprasum*	Amaryllidaceae
Lettuce	*Lactuca sativa*	
Marigold	*Lactuca sativa*, *Tagetes* spp.	Asteraceae
Starflower, borage	*Borago officinalis*	Boraginaceae

Common name	Species	Family
Mustard, Dijon mustard	*Brassica juncea*	Brassicaceae
Chinese kale	*Brassica oleracea* var. *alboglabra*	
Broccoli	*Brassica oleacea* var. *italic*	
Cabbage, red cabbage	*Brassica oleracea* var. *capitata*	
Green cabbage	*Brassica oleracea* var. *gongylodes*	
Kohlrabi	*Brassica rapa* var. *rapa*	
Rapini	*Brassica rapa* var. *rosularis*	
Tatsoi	*Brassica rapa* var. *chinensis*	
Pak Choi, red Pak Choi	*Brassica rapa* var. *niposinica*	
Mizuna	*Eruca sativa*	
Arugula	*Lepidium bonariense*	
Peppercress	*Raphanus raphanistrum* var. *sativus*	
Radish, China rose radish, daikon radish	*Raphanus sativus* var. *longipinnatus*	
Water spinach	*Ipomoea aquatica*	Convolvulaceae
Melon	*Cucumis melo*	Cucurbitaceae
Cucumber	*Cucumis sativus*	
Pea	*Pisum sativum*	Fabaceae
Fenugreek	*Trigonella foenum-graecum*	
Basil, red basil, green basil	*Ocimum basilicum*	Lamiaceae
Lemon balm	*Melissa officinalis*	
Perilla	*Perilla frutescens*	

Common name	Species	Family
Chickpea	*Cicer arietinum*	Leguminosae
Alfalfa	*Medicago sativa*	
Bean, green bean	*Phaseolus vulgaris*	
Fenugreek	*Trigonella foenum-graecum*	
Fava bean	*Vicia faba*	
Lentil	*Lens culinaris*	
Pea	*Pisum sativum*	
Clover	*Trifolium*	
Evening primrose	*Oenothera L.*	Onagraceae
Wheat (wheat grass)	*Triticum aestivum*	Poaceae
Rye	*Secale cereal*	
Popcorn, corn	*Zea mays*	
Buckwheat	*Fagopyrum esculentum*	Polygonaceae
Sorrel	*Rumex acetosa*	

Microgreens are also known as “living superfood/functional food” because they contain chlorophyll, beta carotene, lutein, and large amounts of micro and macro nutrients such as Fe, K, Zn, Ca, N, P, S, Se, Mn, Mo, and Mg (Gupta et al. 2023). Microgreens are an emerging food source, and they contain. Besides these nutrients microgreens are also a rich source of phytochemical compounds, which have an immense potential to increase human health and help in improving disease. Microgreens has the large content of bioactive components such as ascorbic acid, phylloquinone, α-tocopherol, β-carotene, phenolic antioxidants, carotenoids, anthocyanins, glucosinolates, and sugar contents. A comparison of phytochemical concentrations in red cabbage (*Brassica oleracea* L. *var. capitata*) during the microgreen and adult growth stages revealed that the microgreen stage has a high amount of phylloquinone (2.8 g/100 g FW), β-carotene (11.5 mg/100 g FW), and

glucoraphanin (4.8 mol/g DW) compared to the matured stage. Furthermore, anthocyanins are more abundant at the mature stage than at the microgreen stage (Choe et al. 2018).

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3 Microbial Consortia

Microbial consortia contain a varied range of microorganisms with varying environmental habits, such as soil type, and host plant. The microbial consortia have preferred colonization areas, and action against different pathogen species and enhanced plant growth and development (Lopez-Cervantes and Thorpe 2013). Whereas, individual strains of microbes may have various modes of action, combining numerous microorganisms into consortia can widen the nature of their actions towards an extensive variety of plant diseases. The single strains are replaced by microbial consortia or combinations of microbe's products because single strain production cost is higher (Kashyap et al. 2020; Morales-Cedeño et al. 2021). The rationale behind microbial consortia assumes that, under variable environmental conditions, distinct components of the inoculated microbial communities are individually activated by rhizosphere signals and host plant ecophysiological responses to convey their positive impact on plant growth. Examples include the stimulation of phosphate solubilizing microorganisms in a shortage of soluble P forms in soils, the encouragement of P mineralizers after the addition of organic fertilizers, and the promotion of chitinase-producing bacteria in response to harmful fungal proliferation (Kashyap et al. 2020). Moreover, the microbial consortia can also enhance the plant growth and activity of other microorganisms, which are present in nearby soil environments. Microbial consortia consist of a variety of microorganisms including

mycorrhizal fungi, plant growth promoting bacteria (PGPB) and some beneficial microorganisms.

Microbial consortia are two types such as simple and complex. The major difference in both type consortia is in terms of fermentation strategy or protocol, in which microbes are grown either individually or in combination with other strains in a suitable medium (Bashan et al. 2020). Fermentation is an essential stage because a greater number of species often results in a greater number of interactions between strains, leading to production of variations in metabolite secretions. Therefore, the effectiveness of microbial consortia in field conditions is dependent on the types and ability of strains, such as adaptation to adverse climate conditions, persistence in soil and survival (Gosal and Kaur 2017). Additionally, the microbes in the consortia not only compete with others for plant growth promotion, but they also compete for nutrition and space in soil (Ney et al. 2018; Morriën 2016; Pandey et al. 2012).

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4 Interaction Among Microorganisms in Consortia

Microbes generally show three types of interactions (i) positive (ii) negative and (iii) neutral, among each other within a consortium (Singh et al. 2019) (Fig. 1). Positive interaction typically forms a network that assists individual members by cross-feeding, in which a particular bacterium uses the metabolic products created by another consortium member. The positive interaction including mutualism, protocooperation and commensalism. During mutualism each member of a mutualist community depends on the others for survival because they share necessary resources or eliminate toxins from each other's bodies (Roell et al. 2019). Commensalism is a one way- positive interaction, in

which one member benefits while the other is unaffected. In interactions between species that involve protocooperation are advantageous to both species growth rates, they are not necessary for either to survive (Singh et al. 2019). A variety of negative interactions, such as amensalism, predation, parasitism, and competition, reduce the bacterial members within a consortium and undermine the community's structure and ability to function. Amensalism is a kind of unidirectional interaction in which a partner's producing poisonous substances has an impact on one member's ability to grow (Roell et al. 2019; Moënne-Loccoz et al. 2015). Additionally, predation and parasitism are interactions in which the survival of one species is dependent on the consumption of another, resulting in dynamics of populations that frequently show continuous variations. During competition, interacting members of a consortium compete for the same resource, such as nutrients, water, or even space, the faster-growing species wins out over time (Moënne-Loccoz et al. 2015). Neutralism occurs when two species ingest distinct substances (nutritional divergence) and neither produces molecules that are inhibitory to other members of the consortium. In which, members of the consortia do not influence one another during neutral interactions.

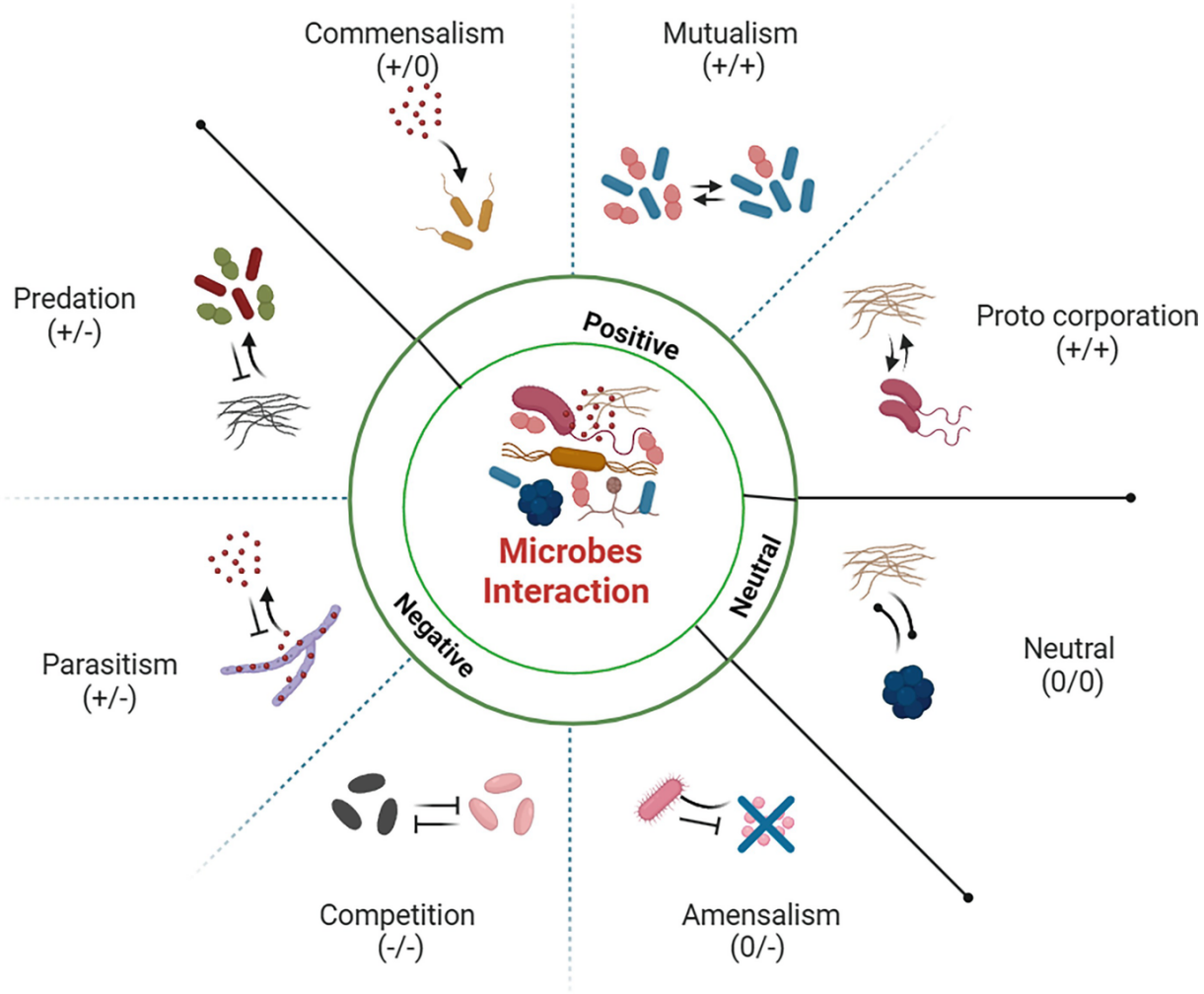


Fig. 1 Interaction among microorganisms in consortia. (Created with BioRender.com)

The mutualistic interaction is more desirable for development of good consortia because in agriculture it is necessary that consortia members should positively interact. The mutualistic interaction provides the steady performance over an extended period of cultivation to produce the anticipated beneficial effect when applied to a crop (Mahmud et al. 2020; Liu et al. 2012). The microbial communication is dependent on the production, detection and response to extracellular signalling molecules, which control the maintenance of the microbial population in consortia. In this condition only compatible microbes are involved in modulating the plant defence response and

affect the plant overall growth and development (Mukherjee and Bassler 2019).

Microbial consortium communication is strongly relying on the molecular signals among the microbes, in which quorum sensing plays a major role in bacterial compliance during consortia development (Liu et al. 2012). Several signal molecules have been reported, which participate in quorum sensing during consortia formulation. Among all, acyl homoserine lactone (AHL) signal molecules are the most well documented in the bacterial population. AHL molecules induce the intracellular Ca^{+2} levels in primary roots as well as they also stimulate the plant biomass, formation of biofilm in root and promote nodulation in plants (Veliz-Vallejos et al. 2014; Pérez-Montaño et al. 2013; Song et al. 2011). Bacteria can transition between two different gene expression programs due to quorum sensing: (i) one at lower density of cells for individual and asocial behaviour, and (ii) other at high cell density for consortia-preferential social and group behaviours (Ng and Bassler 2009). A study conducted by Williams et al. (2007), revealed the quorum-sensing systems react to changes in the number of cells in the following way. Auto-inducers are low-molecular-weight compounds that are generated intracellularly and then passively or actively secreted outside of cells. The extracellular concentration of these compounds increases with the number of cells in a population. At last, the auto-inducers aggregate above the detection threshold, and the similar receptors bind with auto-inducers. This binding process triggers signal transduction cascades, which result in population-wide changes in gene expression. Therefore, quorum sensing allows cells in the group to function in cooperation and act as collectively without affecting the specific function of complementary PGPB in consortium (Altaf et al. 2017; Schikora et al. 2016).

Besides the AHL molecules, the volatile organic compounds (VOCs) produced by bacteria, also act as signaling molecules. They are participating in bacteria-bacteria, bacteria-fungus, and bacteria-plant communication (Schikora et al. 2016). Volatile organic compounds (VOCs) are low molecular weight (<300 Da), high vapour pressure, low boiling point and lipophilic signal molecules such as terpenoids, alkanes, ketones, alcohols, alkenes, and sulphur-containing compounds (Fincheira and Quiroz 2018). These VOCs compounds can have beneficial impacts on the growth of both local or distant bacteria, which alter their behaviour and influence antibiotic resistance. The VOCs compounds influence defense response, hormonal signalling and microbial stress tolerance, virulence and bioformulation related gene expression (Bitas et al. 2013). Additionally, many species of *Pseudomonas* and *Bacillus* produced antimicrobial activity containing volatile organic compounds (VOCs), which are utilized as biocontrol agents against plant diseases (Schulz-Bohm et al. 2017; Villarreal-Delgado et al. 2018). Microbial interaction in a consortium is contingent upon several factors, including (i) a clear and accurate description of the consortium, the taxonomic affiliation of the strains, and identification protocols; (ii) a comprehensive account of the consortium's formation; (iii) a comprehensive declaration of sensitive parameters, including soil physicochemical parameters and the precise dosage administered; and (iv) suitable living populations of each species coexisting, which influences the success of the inoculated consortium (Chávez-Díaz et al. 2020).

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5 Multitude Function of Microbial Consortia in Microgreen Cultivation

Microbial inoculation single or consortia have displayed significant beneficial effects on plant growth and development including direct or indirect (Fig. 2). In direct effect they help in nutrient assimilation, phytohormones production and signaling, p-solubilisation, siderophore production and nitrogen fixation. Whereas indirect include pathogen suppression, inducing defense responses such as ISR and SAR, and reduced the concentration of ethylene in plants (Ali et al. 2020).

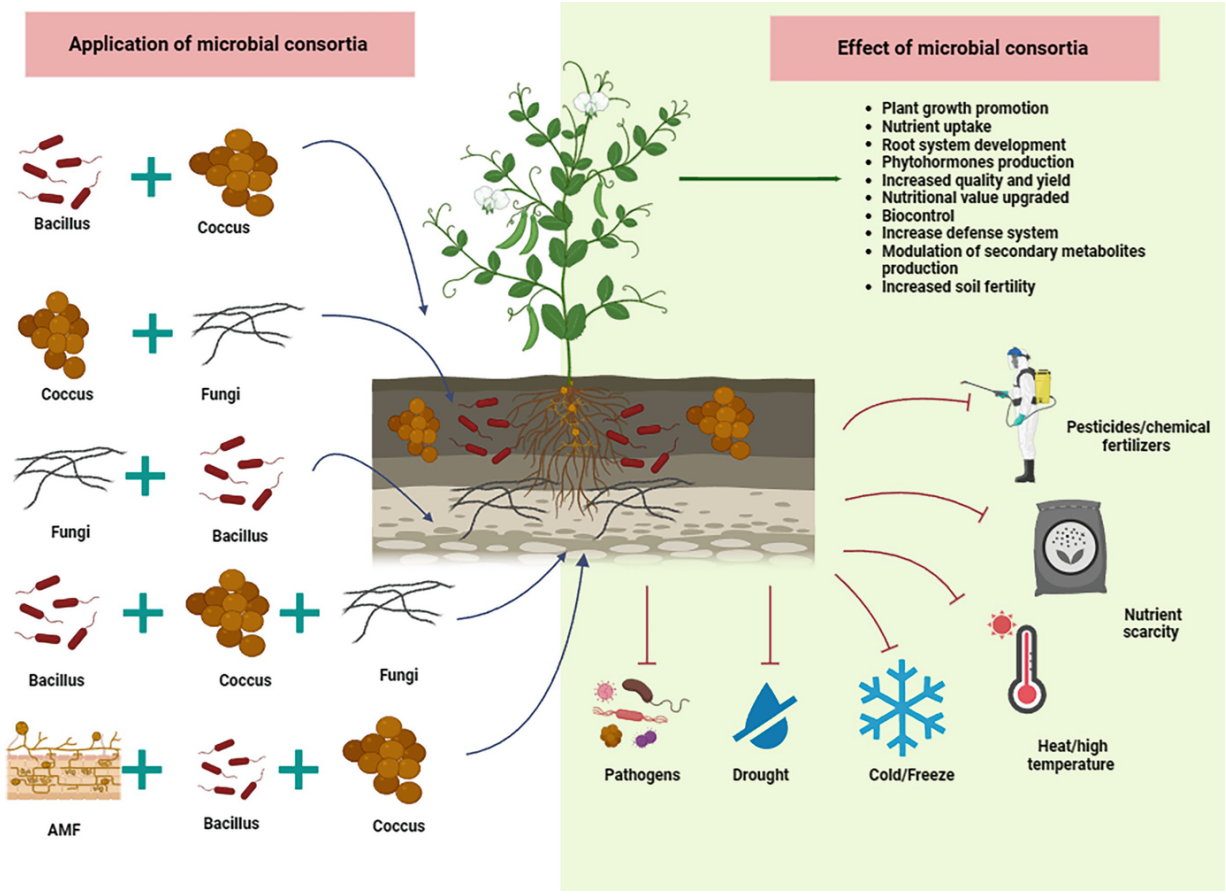


Fig. 2 Schematic diagram showing the combination of different microbial consortia and their effect on plants. (Created with BioRender.com)

Several studies have reported the potential effect of microbial consortia on microgreen cultivation (Guardiola-Márquez et al. 2023). According to Zhao et al. (2015), *Bionectra pityrodes* increased the growth and nutrient

content especially flavonoid (rutin, quercetin) in Buckwheat (*Fagopyrum tataricum*). Moreover, the application of six microbial strains consortia including *Klebsiella* spp., *Nocardia* spp., Rhodococcus spp., *Paenibacillus polymyxa*, and *Paenibacillus polymyxa* improved the growth and nutrient content in Lettuce (Sas-Paszt et al. 2023). Not only abiotic stress amelioration and nutrient acquisition, but the microbial consortia also suppresses the disease causing pathogen growth in microgreen. During the hydroponic culture tank method, the combination of three antagonists *T. viride*, *B. thuringiensis and P. fluorescens* significantly reduce bacterial wilt incidence of Lettuce plant. Additionally, the application of *Lactobacillus*, *Nitrobacter*, *Bacillus* and *Nitrosomonas* has a positive effect on the yield and growth of spinach plants in aquaponic conditions (Khastini et al. 2019). In continuation another study also concludes the same, where *Rahnella* spp., *Nitrosomonas* spp. and *Nitrobacter* spp. have a positive effect on the yield of Lettuce *Lactuca sativa* (Ajijah et al. 2021; Day et al. 2021). Additionally, Rhizobium, phosphobacteria, PPFM combination increased the germination rate and vigour index of black gram, which ultimately uplift the yield of microgreen plant.

Not only abiotic and biotic stresses amelioration, but the microbial consortia also improve the nutrient content in microgreen plants. Therefore, they have the ability to upgrade the plant's nutritional value by increasing their nutrient content and some beneficial compounds. According to Yilmaz (2022), mixed consortia of microbial inoculants improve the yield and essential oil profiling in coriander plants. Similarly, *Trichoderma afroharzianum*, *Azotobacter chroococcum* and 6-pentyl-α-pyrone inoculant increased the rosmarinic acid and photosynthetic activity in Basil plants (Comite et al. 2021). Rosmarinic acid has several health benefits including anti-inflammatory,

antioxidant, and anti-allergic properties, which clearly indicate that application of microbial consortia enhanced the medicinal properties of microgreen plants.

These several reported researchers confirm that microbial consortia have many beneficial effects such as in terms of growth, yield, stress amelioration, nutrient enhancement. This indicates the possibility of using innovative microbial consortia in the microgreen cultivation. Some microbial consortia and their effect on plants were mentioned in Table 2.

Table 2 Some microbial consortia and their effect on microgreen cultivation

S. no.	Consortia	Effect	Crop	Reference
1.	*Klebsiella* spp., *Nocardia* spp., *Rhodococcus* spp., *Paenibacillus polymyxa*, and *Paenibacillus polymyxa*	Improved growth parameters (plant height, length, biomass) and nutrient content	Lettuce	Sas-Paszt et al. (2023)
2.	*Lactobacillus*, *Nitrobacter*, *Bacillus* and *Nitrosomonas*	Improved plant growth and yield	Spinach	Khastini et al. (2019)
3.	*Klebsiella*, *Pantoea*, and *Enterobacter*	Degrade soil hydrocarbon contaminants and restoring their natural fertility by stabilizing the soil carbon: Nitrogen ratio	Chickpeas, coriander, fenugreek, and spinach	Poddar et al. (2023)
4.	*Rahnella* spp., *Nitrosomonas* spp., and *Nitrobacter* spp.	Enhanced yield and growth of spinach plant in aquaponic condition	*Lactuca sativa*	Ajijah et al. (2021) and Day et al. (2021)
5.	*Ensifer meliloti*, and *Rhizobium leguminosarum*	Promote seed germination and growth, antagonistic against *Fusarium oxysporum*	Fenugreek	Kumar et al. (2011)

S. no.	Consortia	Effect	Crop	Reference
6.	*Trichoderma afroharzianum*, *Azotobacter chroococcum*	Increased the rosmarinic acid and photosynthetic activity	Basil	Comite et al. (2021)
7.	*Rhizobium*, phosphobacteria, PPFM	Increased germination and vigour index	Black gram	Raja et al. (2019)
8.	*Acinetobacter* sp., *Aeromonas* sp., *Alcaligenes* sp., *Bacillus cereus*, *Bacillus subtilis*, *Candida* sp., *Clostridium* sp., *Lactobacillus* spp., *Listeria monocytogenes*, *Micrococcus* sp., *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Pseudomonas putida*, *Saccharomyces cerevisiae*, *Salmonella* sp., and *Yarrowia lipolytica*	Increased surface area and length of root	Cabbage	Cobos-Torres et al. (2021)
9.	*Azotobacter*, *Bacillus* sp.	Enhanced germination percentage and number of leaves, chlorophyll content, fresh and dry weight	Radish	Nandish et al. (2021)
10.	*Bacillus cereus*, *Bacillus altitudinis*, *Comamonas* and *Stenotrophomonas maltophilia*	Increased antioxidant enzymes activity i.e. SOD, POD and uptake of nutrients i.e. Ca, Mg, K, Na, Fe, Cu and Zn	Alfalfa	Shahzad et al. (2020)
11.	*Azospirillum brasilense*, *Gluconacetobacter diazotrophicus*, *Herbaspirillum*	Ability to produce auxins, phosphate solubilizing capability and	Carrot	Pellegrini et al. (2021)

S. no.	Consortia	Effect	Crop	Reference
	seropedicae, and *Burkholderia ambifaria*	ACC deaminase activity		

6 A Case Study of Use of Microbial Consortium in Microgreen Cultivation

Plants associated with microbial diversity are the result of ecological and evolutionary events (Singh et al. 2020). In traditional agriculture, plant growth-promoting microorganisms (PGPMs) or microbial consortia have been widely employed to improve soil-based farming's phytoremediation efficacy, plant development, and resistance to environmental stress. There are lots of opportunities to employ microbial consortia to their fullest because hydroponics and vertical farms have a regulated environment (Dhawi 2023). In a study, inoculation of bacterial strain *Herbaspirillum* sp. ST-B2 markedly enhanced the growth of common Buckwheat sprouts and microgreens. The highest effect of seed inoculation with bacterial endophyte on sprouts growth was found in treatment of 20% (v/v) inoculum while 10% (v/v) inoculum (1.0×10^{-7} CFU mL^{-1}) had the positive effect on growth of microgreens (Briatia et al. 2017). An increasing number of experimental studies have indicated that Brassicaceae plants can form mutualistic interactions with non-mycorrhizal fungi such as those in the genera *Serendipita* (syn. Piriformospora), *Cladophialophora*, *Periconia*, *Microdochium*, and *Colletotrichum* (Hiruma et al. 2018). A study on *Chlorella vulgaris* and three symbiotic bacterial strains found that the symbiotic bacterium *Pseudomonas* sp. had a growth-promoting effect on *C. vulgaris*, while the other two inhibited it. The interactions between *C. vulgaris* and *Pseudomonas* sp. were investigated under different

cultivation conditions. The study found that the chlorophyll content in *C. vulgaris* cells was higher in co-culture than in single algal culture, and the algal cells showed higher photosynthetic efficiency in photoautotrophic conditions (Guo and Tong 2014). Three endophytic bacteria from sugar beet roots have been reported to produce indole-3-acetic acid (IAA) *in vitro*, promoting plant growth. Inoculation of these bacteria increased plant height, weight, leaves, and phytohormone levels. The bacteria were recovered from healthy beet roots up to 8 weeks after inoculation, suggesting they may be a habitat (Wetzel et al. 2010).

A study on sweet basil showed treatments increased basil yield, photosynthetic activity, and secondary metabolite production. The use of these bioformulations can lead to farming systems with minimal environmental impact, improving yield and quality in fresh basil cultivation (Shi et al. 2009). The arbuscular mycorrhizal fungi (AMF) generate substantial improvement in plant growth, with positive impact on nutrient uptake and systemic resistance to fungal diseases and other pests. These benefits create important and economically viable alternatives for conventional methods of cultivation (Shi et al. 2009). Moreover, *Trichoderma harzianum*, an opportunistic symbiotic fungus, produces active elicitors against plant pathogens and insects and promotes plant growth (Baum et al. 2015). Edible flowers and basil, commonly used fresh herbs, have been linked to food-borne illnesses. A study comparing microbial diversity between organic and conventional growing methods found that organically grown samples had higher microbial diversity than conventionally grown samples (Sharma et al. 2017).

7 Application Methods and Techniques of Microbial Consortia in Microgreen Cultivation

Currently, the usage of creative agricultural solutions like hydroponics and vertical farming is growing due to the need of maintaining soil condition because of the expense of fertilizer, pesticides, and climate concerns. The urban version of hydroponics known as “vertical farming” (Schnitzler 2013; Despommier 2013) provides ways to save water and land, boost crop productivity, and reduced the need of pesticides and soil fertilizers (Kaur and Chawla 2021; Kulak et al. 2013). Consequently, vertical farming is seen as a groundbreaking approach to addressing climate change concerns and a quality enhancement (Kulak et al. 2013). However, as indicated by the fact that this technology is only being utilized to grow a small number of crops, including microgreens (like Bok choy, and Radishes) at 6%, leafy greens (like lettuce) at 57%, flowers at 10%, tomatoes at 16%, and herbs at 11%.

Depending on the use of nutrient solutions and the root structure of the plant, most current hydroponic systems are divided into seven main types: absorption systems, wave, and flow systems (flood and flow), drip irrigation, nutrient film technology (NFT), and deep aquaculture, aeroponics and aquaponics (Kumar and Ge 2020). Unlike the soil-based system, the hydroponic system provides a controlled and ideal environment for the extraction of higher concentrations of antioxidants (Barker and Koenning 1998). The soil system supports plants and the growth environment through the colonization of PGPM and other lower animals, such as earthworms, which contribute to nutrient cycling (Zapata-Vahos et al. 2020). Traditional hydroponic, aquaponic or aeroponic horizontal layers are stacked on top of each other to create a vertical framework

in vertical farming systems (Nair et al. 2015). Several studies have demonstrated that bacterial consortium inoculation has a more significant effect on plant development and aids in the reduction of biotic and abiotic stress (O'Callaghan 2016). For example, combining *Paenibacillus polymyxa* and *Bacillus megaterium* with *Rhizobium* has been shown to increase the biomass of *Phaseolus vulgaris* plants compared to *Rhizobium i*noculation alone (Kumar and Ge 2020). In addition, N, K and Na concentration, biomass and yield were improved by inoculating legumes with *Rhizobium* and *Pseudomonas* bacteria. Following inoculation with a combination of *Bradyrhizobium* and *Streptomyces griseoflavus*, *Glycine max* showed increases in nodulation, nitrogen fixation, and nutritional absorption. According to Wiggins et al. (2020), the use of multiple substrates with lower fertilizer rates showed promising results in the productivity of lettuce varieties, showing how hydroponics or vertical cultivation can incorporate PGPMs. To overcome the problems associated with soilless systems, PGPMs for hydroponics or vertical cultivation should be presented as selection criteria. These criteria include high competitiveness with substrate microorganisms, stability under changes in environmental conditions, and effective root system colonization. Plant resistance may be increased by choosing microorganisms that are anti-pathogen (Stouvenakers et al. 2019). To prevent contamination, physical sterilization techniques such as UV light should be considered when adding PGPM substrates to hydroponic systems (Varia et al. 2022). According to these studies, inoculating seedlings with a microbial suspension boosted growth and the overall biomass of tomato, *Glycine max* (L.) Merr., *Banana, berangan*, and *Triticum aestivum* (Ye et al. 2020; Paradiso et al. 2017). Furthermore, plants like Lettuce and Strawberries benefited from the use of biostimulant

extracts or siderophores added to the medium in a soil-free system (Sambo et al. 2019; Gravel et al. 2006). Thus, to maximize their beneficial effects, PGPM application modes must be defined in accordance with various soil-free systems. Moreover, by decomposing organic matter, lowering the accumulation of dangerous materials, and aiding in pH balance, the application of PGPM in a soil-free system can enhance the quality of recycled water. As a result, recycled water is used more effectively because it doesn't need to be changed or replaced as often and can be used again. Plant health and water quality both can be preserved by the presence of PGPM. All things considered, using PGPM in a soil-free system has a lot of advantages and can improve the sustainability and effectiveness of hydroponic and vertical farming practices. Table 3 presents our recommended PGPM application mode based on the plant root system's susceptibility to pathogens and elevated humidity. In addition, seed or seedling pretreatment or inoculation with a microbial suspension is recommended for all soil systems except drip irrigation systems.

Table 3 Types of hydroponics and suggested plant growth promoting microbe (pgpm) mode

S. no.	Hydroponic types	Definition	Suggested PGPM mode application
1.	Wicking system	Small-scale producing plants (such as leafy greens and herbs)	Microbial suspension inoculation of seedlings prior to treatment
2.	Flood and drain: Ebb	Commercial settings for the growing of seedlings	Microbial suspension inoculation of seedlings prior to treatment
3.	Drip watering	Larger fruiting crops including tomatoes, cucumbers, peppers, and strawberries are produced commercially	Align the course of treatment: Addition of bio stimulant extract to hydroponic medium
4.	Nutrient film method	Utilized commercially for green vegetables like lettuce	Microbial suspension inoculation of seedlings

S. no.	Hydroponic types	Definition	Suggested PGPM mode application
			prior to treatment
5.	Submerged water civilization	For tiny leafy plants like lettuce or herbs, deep water culture techniques are utilized in commercial settings	Microbial suspension inoculation of seedlings prior to treatment
6.	Aeroponics	Commercial producers of tiny, leafy plants and mini-tubers made of potatoes	Microbial suspension inoculation of seedlings prior to treatment
7.	Aquaponics	Growing fish and plants in the same system	Inoculation of plants with microbial suspension before treatment

8 Conclusion

Application of innovative microbial consortia containing beneficial microorganisms and bioactive metabolites is being explored to improve agricultural production and resource use efficiency. They can inhibit plant pathogens, induce systemic resistance, and promote plant growth, which provide better growth and development in plants during stress conditions including both biotic and abiotic. In conclusion, the use of microbial consortia approach harnesses the power of microbial communities to enhance microgreen cultivation, promising sustainable and resilient agricultural practices. By fostering synergistic relationships between plants and microbes, we pave the way for increased yields, improved nutrient uptake, and reduced reliance on conventional fertilizers. As we delve into this new era, the potential for eco-friendly and resource-efficient microgreen production becomes not only a reality but a beacon of hope for a more sustainable and environmentally conscious future in agriculture.

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Bio-stimulating Role of Plant Growth Promoting Microorganisms in the Sustainable Production of Micro Greens

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Abbreviations

PGPM Plant growth promoting microorganism

AMD Age-related macular degeneration

RH Relative humidity

GDL Gas discharged lamps

LEDs Light-emitting diodes

AMF Arbuscular mycorrhizal fungi

Authors Meenakshi Sanyal and Debjanee Chowdhury are joint first author.

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1 Introduction

Tender young seedlings of vegetables and herbs which are full of different components with nutritive values are called microgreens. Microgreens are a leading food choice in the twenty-first century because of which microgreens are now being cultivated in small scale as well as in large scale these days. People incorporate numerous types of microgreens in their daily diet. These microgreens are home to various families such as Amaranthaceae (amaranth, beetroot, quinoa, spinach, buckwheat, thistle), Amaryllidaceae (leek, garlic, onion), Apiacaceae (parsley, carrot, fennel, celery, dill, cherry, coriander, coriander), Boraginaceae (phacelia), Asteracea (lettuce, chicory, radicchio, endive-tarragon, common dandelion), Cucurbitaceae (melon, cucumber, pumpkin), Malvaceae (jute mallow/Nalta jute) Brassicaceae (radish, arugula,

chicken, watercress, watercress). -cabbage, chicory, wild rocket), Convolvulaceae (watercress), Poaceae (corn, lemongrass), Lamiaceae (chia), Onagraceae (night rain eguminosae (chickpea, alfalfa, broad bean, green bean, fenugreek, clover, pea) and Portulacaceae (common purslane, muskrose) (Bhaswant et al. 2023).

These variety of families of microgreens differ on the basis of days of sowing, secondary metabolites, shelf life, colour, texture, taste and many other features. Amaranthaceae family offers microgreens such as amaranth, quinoa, and beet as well, which have a distinct earthy taste and bright color palette. Leafy greens are prized for their high concentration of minerals, vitamins, and especially betalain pigments (Belošević et al. 2024). In addition to red betalains that bring the beet microgreens color researchers have been concentrating on additional possible health advantages including anti-inflammatory and antioxidant properties (Lone and Pandey 2024).

It is doubtless that Brassicaceae family is the rivet in this world for peppery microgreens. Along with arugula, radish, broccoli, and cabbage are some microgreens that are also used popularly in salads, being renowned for their power flavour which is concentrated in them compared to the mature ones (Bhaswant et al. 2023). These microgreens contain an abundance of these vitamins, minerals and antioxidant, and as for broccoli microgreens, they are particularly rich in certain sulforaphane compound that provides anti-cancer effect (Lone and Pandey 2024). The family Apiaceae is the source of a wide range of microgreens that can add taste value to any dish. Dill microgreens are not only noted for their light dill flavour but also as an option for seafood dishes. Carrot microgreens, on the other hand, add a sort of sweetness to it. The investigation of precise connections between their character and the level of utilization across the east is yet

in the process, but those bioactive compounds could be supportive in the microgreen field as well.

With all the amazing mix of Asteraceae, diners really get a colorful palette. People often use sunflower sprouts, lettuce and chiles in salads as they are rich in flavour. Asteraceae plants synthesize plenty of phytochemicals; hence microgreens of their species must contain some of these compounds (Ojo et al. 2022). Research, therefore, becomes necessary to identify the microgreens phytochemicals. Malvaceae microgreens such as jute mallow are drawing a silver lining. Though not enough research has been done on the subject, what is known about these species suggests that they may contain beneficial chemicals and have antioxidant activity (de Oliveira et al. 2012).

Observing these various properties exhibited by microgreens for agricultural as well as commercial purpose (Bhaswant et al. 2023). Each of these types of microgreens are unique in their own ways but collectively they form a big nutritious food source which turns to be a wonderful choice for a person willing to take a health-conscious choice. The microgreens come in a range of colours and tastes and also have loads of vitamins and possible health perks. From the peppery kick of Brassicaeae microgreens to earthy richness of Amaranthaceae greens, the nutty crunch of Asteraceae shoots to the aromatic allure of Lamiaceae herbs, microgreens are the ones that captivate the senses and nourish the body with their generous goodness. With the continuous investigations of health benefits and culinary applications of microgreens, these little plants are definitely leading the way to the nutritional and gastronomic future.

The bio-stimulating role of plant growth-promoting microorganisms (PGPMs) is increasingly recognized as vital tool in sustainable agriculture, particularly in microgreens production. PGPMs, including beneficial bacteria, fungi,

and other microorganisms inhabiting the rhizosphere, promote plant growing through various mechanisms. In microgreens cultivation, harnessing the bio-stimulating properties of PGPMs offers numerous benefits. Firstly, PGPMs enhance nutrient uptake and assimilation by microgreens, leading to healthier growth and higher nutritional content. They also stimulate root development, improving overall plant vigour and yield, crucial for maximizing productivity in microgreens production (Lopes et al. 2021). Additionally, PGPMs enhance microgreens' resilience to environmental stresses and reduce the need for chemical pesticides and fungicides, promoting environmental sustainability and food safety. Moreover, PGPMs contribute to soil health and fertility by promoting organic matter decomposition and improving soil structure. Incorporating PGPMs into microgreens production through various strategies can enhance productivity, reduce environmental impacts, and promote the nutritional quality of microgreens, contributing to a more sustainable food system.

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2 Mineral Content of Microgreens

Microgreens are filled with nutrition, containing up to 40 times more concentrated nutrients than their mature counterparts. Research have found that the nutritional content of microgreens can vary significantly conditional on the variety and growing conditions (Cardarelli et al. 2024). That being said, microgreens tend to be rich in vitamins A, C, E, and K, as well as iron and calcium (Alloggia et al. 2023). The nutrient levels depend on the type of green being grown. Red cabbage microgreens contain high levels of vitamin C, while kale microgreens are rich in iron and calcium. On the other side, there are microgreens of versatile varieties like brassica broccoli which contains high concentration of sulforaphane a compound that boosts

the immune system and fights cancer cells and prevents formed by inflammation.

Microgreen are packed with phytochemicals and antioxidants that can have numerous health benefits. Phytochemicals are compounds found in plants that provide many of the protective properties of fruits and vegetables. These compounds, such as polyphenols, flavonoids, and carotenoids, help keep cells from damage by neutralizing free radicals. Free radicals can lead to oxidative stress, which is linked to several chronic diseases. Microgreens are also rich in antioxidants, which aid in shielding cells from harm brought on by free radicals. Antioxidants can be found in many plant foods, but microgreens tend to have higher levels than their mature counterparts (Renna et al. 2020). Research has revealed that some varieties of microgreens contain up to nine times more antioxidants than the mature version of the same plant.

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3 Applications of Microgreens

Microgreens, the young and tender shoots of various vegetable, herb, and grain plants, are becoming more and more well-liked in the culinary and nutritional sectors since they are not only aesthetically pleasing but also very nutritious. These miniature greens are versatile ingredients, adding both flavour and visual appeal to a wide range of dishes. Microgreens are aesthetically pleasing, but they are also a great source of vital nutrients including vitamins, minerals, and antioxidants (Tan et al. 2019). They are perfect as salad, soup, sandwich, and other culinary creative garnishes because of their rich tastes. Additionally, studies on the possible health advantages of microgreenssuch as boosting cardiovascular health, lowering inflammation, and enhancing immunological function—are being conducted (Kumar and Goel 2019). Additionally, their rapid growth cycle and ability to be

cultivated in small spaces make them accessible for home gardeners and urban farmers, contributing to sustainable food production practices. Overall, the applications of microgreens extend from gourmet cuisine to health-conscious dietary choices, highlighting their versatility and nutritional value (Fig. 1).

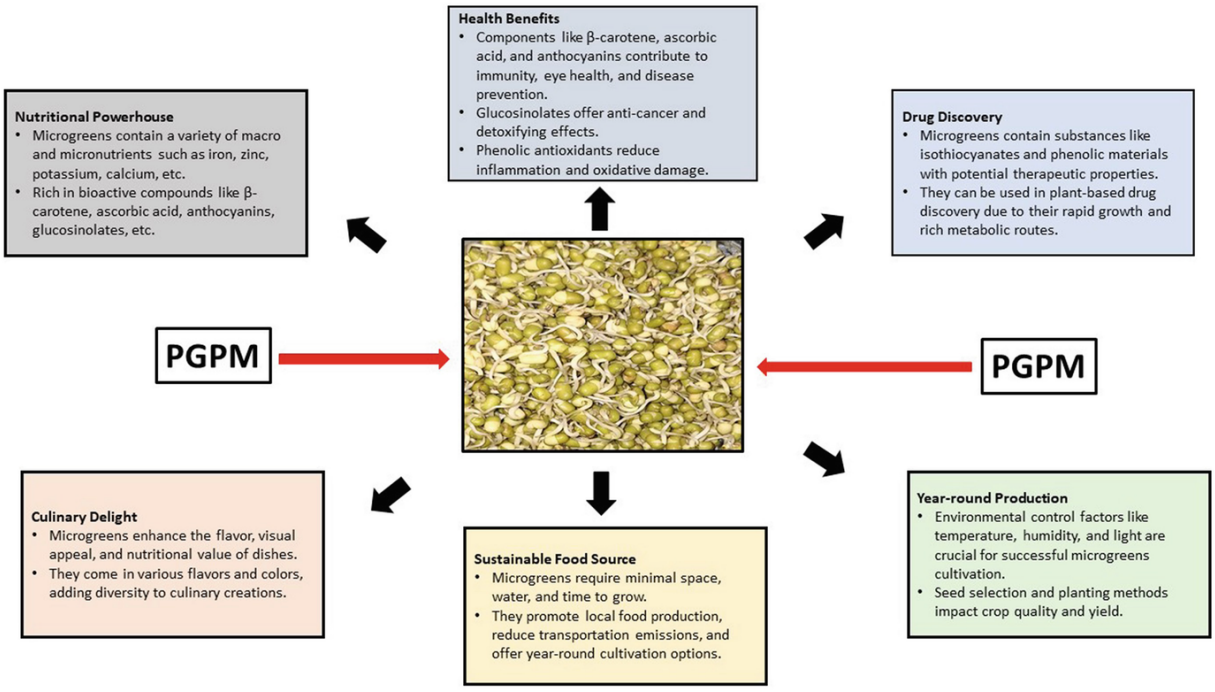


Fig. 1 The figure illustrates the diverse applications of microgreens in various fields, including culinary, nutraceutical, and agricultural industries. Microgreens, tender young seedlings of vegetables and herbs, are depicted in different stages of growth, highlighting their visual appeal and nutritional content. The figure demonstrates how microgreens are utilized as garnishes in gourmet dishes, incorporated into salads and sandwiches for added flavour and texture, and used as functional ingredients in health-focused cuisines. Additionally, the figure showcases the potential role of microgreens in nutraceutical discovery, emphasizing their rich nutrient profile and health benefits. Furthermore, the figure highlights the application of plant growth-promoting microorganisms (PGPMs) in sustainable microgreens production, illustrating how these beneficial microorganisms enhance biomass production and nutrient uptake in microgreens cultivation. Overall, the figure underscores the versatility and potential of microgreens in contributing to a sustainable and nutritious food system

3.1 Microgreens as Nutritional Powerhouse

Microgreens are edible plants with macro and microelements, like iron, zinc, potassium, calcium, nitrogen, phosphorus, sulfur, manganese, selenium, molybdenum, and others. They're also full with plant phytochemicals that may impact human health and potentially cure diseases. Microgreens supposedly have very elevated levels of bioactive substances such as β-carotene, ascorbic acid, anthocyanins, glucosinolates, phylloquinones, α-tocopherol, lutein, zeaxanthin, and phenolic antioxidants (Bhaswant et al. 2023).

3.2 Microgreens Provide Health Benefits

The red and orange colour in fruits and vegetables is resulting from a pigment known as β carotene. It is an organic compound which helps our body make Vitamin A (Polcz and Barbul 2019), which is absolutely essential for immunity, eyesight and growth. Unlike animal sources, plant beta-carotene doesn't let vitamin A levels get too high and toxic. This pigment also acts as an antioxidant. It shields cells against disturbing molecules called free radicals that cause severe damage linked to various cancer and many more (Dawson 2000).

Microgreens have remarkably greater concentrations of ascorbic acid concentration in comparison to the mature plants (Bhaswant et al. 2023). As the essential antioxidant, ascorbic acid neutralizes deleterious free radicals implicated in the making of chronic diseases that also destroy cells (Thiagarajan and Manikandan 2013). Ascorbic acid also helps in building a stronger immune system by helping human body fight diseases more efficiently as well enhances wound healing. It is also essential for the synthesis of collagen.

Several microgreens exhibit hues of red, purple and blue on account of pigments called anthocyanins, but these pigments do not exist only for aesthetic reasons. They can hinder free radicals from causing such damage to the cells

and are thus useful as antioxidants which are related with chronic diseases (Li et al. 2013). They improve cognitive performance along with reduce inflammation (Martins et al. 2023). Nonetheless, mainly cruciferous microgreens that are well known like broccoli and kales have phenolic compounds popularly known as glucosinolates; which are organic compounds with the composition of sulphur in it. They give isothiocyanates, one can consider it as the beneficial compound, when they are chewed or chopped. The probability of getting anti-cancer and detoxifying effects from these compounds is very encouraging (Ibrahim et al. 2023).

Vitamin K significantly puts a stop to unwanted bleeding and helps in the healing process (Dowd et al. 1995). It also plays a consequential role in calcium regulation thereby helping in maintaining strong bones. Vitamin K therefore helps in prevention of osteoporosis (Mladěnka et al. 2022). According to study, microgreens have a higher concentration of Vitamin K than leafy greens (Bhabani et al. 2024). Because it supports healthy bones and blood clotting, including a wide range of microgreens that are high in phylloquinone into our diet may be a better option than other foods.

Microgreens contain an important chemical called α-tocopherol. Many body processes need it. These include nerve signals, muscle movements, immune system activity, preventing free radical damage, and other vital functions (Bhaswant et al. 2023).

The microgreens have antioxidants called phenolic compounds. These special molecules stop oxidation. Oxidation causes damage from free radicals. Inflammation is also reduced by phenolics (Leyva-Jiménez et al. 2019). Other antioxidants affect taste, smell, and colour too. Additionally, it was noted that the microgreens' overall phenolic content varied, especially in broccoli, which had a

ten-fold increase above that of its mature counterparts and sprouts (Bhaswant et al. 2023).

Amazingly, microgreens pack much more lutein and zeaxanthin than grown plants (Gupta et al. 2023). Microgreens like kale, spinach, and cilantro have tons of these nutrients Lutein and zeaxanthin block harmful blue light, shielding retinal cells. This stops age-related macular degeneration (AMD) (Mrowicka et al. 2022).

3.3 Microgreens in Drug Discovery

First of all, microgreens could be mentioned as the extraordinary suppliers of a plenty of different therapeutically very productive substances. Such naturally occurring substances as isothiocyanates, phenolic materials and flavonoids exhibit anticancer, antibacterial, anti-inflammatory and antioxidant properties (Bhabani et al. 2024). Nonetheless, they can be used as a basis of new therapeutic approaches that are very powerful as they have drastic biological effects.

Besides that, microgreens have built-in metabolic routes that create secondary metabolites some of which can be used in drugs hopefully in the near future. Researchers may successfully design microgreens in order to enhance the yield or to change the profile of the valuable substances by taking into the consideration the biosynthetic pathways and regulatory mechanisms of the advancement of the natural compounds in these plants.

Additionally, to this, microgreens have its own antibacterial characteristics (Lone and Pandey 2024). Some microgreen species employ independent defence strategies like the production of phytoalexins, antimicrobial peptides, and other protective chemicals by themselves (Galieni et al. 2020).

3.4 Microgreens as a Culinary Delight

Microgreens, small in size can bring huge enhancement of flavours to any dish. Theyare visually appealing which can be easily understandable from the variety of colours they range from. Along with their immaculate range of tastes, microgreens are a wonderful addition to any food dish. Microgreens have a wide variety of flavours. Arugula microgreens taste peppery in salads. Pea shoots are sweet. Sunflower microgreens are nutty. Basil microgreens taste like herbs. There are many flavours for us to try. Microgreens look colourful on your plate. Green, red, and purple hues abound. Adding microgreens makes dishes look nice. This visual aspect appeals to people. Microgreens can change how dishes look and taste in many ways, not just salads. They make soups, pizzas, curries look better. Plus, they add new flavours. We can place microgreens in sandwiches. They taste good and change the texture. Some microgreens like wheatgrass work well in smoothies. They pack nutrients and turn drinks green. Microgreens in general make food taste better and add more nutritional value to the food (Renna et al. 2020).

3.5 Microgreens as a Sustainable Food Source

Microgreens, those miniature marvels of flavour and nutrition, are not just a fad. They hold immense potential for revolutionizing our food system towards greater sustainability. Microgreens thrive in small spaces, vertical farms, or even repurposed containers into your own little farms. For cities with limited land, this is revolutionary. The University of Connecticut emphasizes that urban gardening, which is essential for feeding our growing city population, is best suited for microgreens (Du et al. 2022). Microgreens are generally cultivated in an environment that is carefully managed with some of the methods like hydroponics or aeroponics that decrease the water lost through evaporation (Moraru et al. 2022). The twenty-first century needs a super-fast crop and that’s what

microgreens are great at. Their maturing time is equally swift with few completing their cycles over 7–21 days. This is also the quickest production cycle which ultimately leads to more several yields from the same technological application (Teng et al. 2023).

3.6 Year-Round Production of Microgreens

In the year-round production approach, it covers the method of breeding and growing a crop or rearing animals all year around, not allowing the natural seasons to disrupt the production process. Microgreens which are nutrient-close seedlings deliver a novel possibility of year-round production resulted from their short life (7–28 day) and also very simple and minimal space. Their flexibility eases them along both the home growers as well as the commercial ventures (Bhaswant et al. 2023). Achieving year-round success with microgreens cultivation hinges on maintaining a controlled indoor environment with stable temperature, humidity, and light levels, which are crucial for steady growth. Microgreens grow best in temperatures ranging from 60 to 70 °F (15–21 °C) and should be watered sparingly. Germination and maintaining warm during favourable season can be carried out by using heat mats (Turner et al. 2020). The researchers investigated the influence of relative humidity (RH) regarding the growth and quality of microgreens. They discovered that maintaining optimal humidity levels between 60% and 70% resulted in increased germination rates, enhanced growth, and improved nutritional content in microgreens. Higher humidity levels facilitated faster growth, reduced water stress, and minimized wilting (Abaajeh et al. 2023). The quality of light affects various aspects of the plant’s growth and phytochemical content. In the past, growers supplemented natural lights with gas discharged lamps (GDL) but these days, light-emitting diodes (LEDs) are becoming crucial in the horticulture industry as

technologies to replace natural light are advancing (Abaajeh et al. 2023).

Seed selection is a crucial step in agriculture and gardening as it directly impacts the quality, yield, and resilience of the crops. The right seed selection can lead to improved resistance to pests and diseases, better adaptation to local climate and soil conditions, and higher nutritional value in the harvested produce. Additionally, selecting seeds from strong, healthy plants can help maintain and improve overall crop quality from one generation to the next (Pathak et al. 2023).

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4 Plant Growth Promoting Microorganisms (PGPMs)—Beneficial Microbes

The small area of soil that surrounds a plant's root system, known as the rhizosphere, is a centre for the activity of microbes in the soil (Cantó et al. 2020). A vast variety of microbial taxa, including prokaryotes (bacteria, viruses, and archaea) and eukaryotes (fungi, oomycetes, nematodes, protozoa, algae, as well as arthropods), inhabit the rhizosphere. Of these, the most prevalent groups are bacteria and fungi, which perform essential ecological roles (Kalam et al. 2017a, b; Buée et al. 2009). Plant growth promoting rhizobacteria, a name first used by Kloepper and Schroth in 1978 (Dutta and Podile 2010), are free-living soil bacteria that flourish in the rhizosphere, actively colonize plant roots, and promote plant development.

This diverse collection of bacteria, which is an essential portion of the soil microbiome, is believed to manufacture and secrete a diversity of regulatory compounds in the vicinity of plant roots, which help to promote plant development (Khoshru et al. 2020; Ahemad and Kibret 2014). PGPMs defend against phytopathogenic

microorganisms, boost nutrient uptake by host plants, and increase resilience to a range of abiotic stressors, all of which have an impact on the general health of plants (Backer et al. 2018; Parray et al. 2016). Various PGPM strains have the ability to boost crop yields, demonstrate biocontrol, improve resistance to foliar diseases, encourage nodulation within legumes, and accelerate seedling emergence (Vejan et al. 2016; Kalam et al. 2020; Swarnalakshmi et al. 2020). Acinetobacter, Aeromonas, Agrobacterium, Allorhizobium, Arthrobacter, Azoarcus, Azorhizobium, Azospirillum, Azotobacter, Bacillus, Bradyrhizobium, Burkholderia, Caulobacter, Chromobacterium, Delftia, Enterobacter, Flavobacterium, Frankia, Gluconacetobacter, Klebsiella, Mesorhizobium, Micrococcus, Pantoea, Pseudomonas, Rhizobium, Serratia, Streptomyces, Thiobacillus, and other genera are among those reported PGPRs (Vessey 2003; Goswami et al. 2016; Ankati and Podile 2018). Table 1 provides a summary of all the many Phyto-beneficial effects of PGPMs.

Table 1 Some PGPMs and their beneficial effects on different plants

PGPM Strain(s)	Tested plant(s)	Effect	Reference
Pseudomonas fluorescens DR11, *Enterobacter hormaechei* DR16, *Pseudomonas migulae* DR35, *Bacillus subtilis*, *Achromobacter piechaudii* ARV8, *Phyllobacterium brassicacearum*, *Paenibacillus polymyxa*, *Rhizobium tropici*, *Azospirillum brasilense*	Foxtail millet (*Setariaitalica* L.), Maize (*Zea mays* L.), Bean (*Phaseolus vulgaris* L.), *Arabidopsis thaliana*, Tomato (*Lycopersicum esculentum* Mill cv. F144), Pepper (*Capsicum annuum* L. cv. Maor), Wheat (*Triticum aestivum* L.)	Drought stress tolerance	Timmusk et al. (2014), Niu et al. (2018), Ilyas et al. (2020), De Lima et al. (2019), Bresson et al. (2013), Figueiredo et al. (2008) and Yang et al. (2009), Rocha et al. (2023)

PGPM Strain(s)	Tested plant(s)	Effect	Reference
Bacillus pumilus, *Exiguobacterium oxidotolerans*, *Bacillus megaterium*, *Azospirillum* sp., *Achromobacter piechaudii*, *Eneterobacter* sp. PR14	Brahmi (*Bacopa monnieri* L.), Maize (*Zea mays* L.), Lettuce (*Lactuca sativa* L.), Tomato (*Lycopersicum esculentum* Mill.), Rice (*Oryza sativa* cv. Sahbhagi), Sorghum (*Sorghum bicolor*), Finger Millets (*Eleusine coracana*)	Salinity stress tolerance	Sagar et al. (2020), Mayak et al. (2004), Bharti et al. (2013), Marulanda et al. (2010) and Fasciglione et al. (2015)
Paenibacillus xylanexedens, *Bacillus amyloliquefaciens*, *Streptomyces* sp., *O. intermedium*, *P. lentimorbus*, *Pseudomonas* spp.	Wheat (*Triticum aestivum* L.), Rice (*Oryza sativa*), Pine (*Pinus taeda* L.), Tomato (*Lycopersicum esculentum* Mill.)	Biotic stress tolerance	Verma et al. (2016), Srivastava et al. (2016), De Vasconcellos and Cardoso (2009), Gowtham et al. (2016), Khan et al. (2012) and Reshma et al. (2018)
Pantoea sp. S32, *Paenibacillus polymyxa*	Rice (*O. sativa* L.), Habanero pepper (*C. chinense*)	Nutrient absorption	Chen and Liu (2019), Pii et al. (2015) and Castillo-Aguilar et al. (2017)
S. marcences, *P. fluorescens*, *Azospirillum lipoferum*, *P. putida*, *B. subtilis*, *Providencia* sp., *Brevundimonas diminuta*	Maize (*Z. mays* L.), Wheat (*T. aestivum* L.)	Seed germination	Almaghrabi et al. (2014), Nezarat and Gholami (2009) and Rana et al. (2011)
Azospirillum lipoferum, *B. subtilis*, *A. protophormiae*, *D.*	Rice (*O. sativa* L.), Tomato (*S. lycopersicum* L.),	Phytohormone(s) production	Kalam et al. (2020), Cassán et al. (2001), Tahir et al.

PGPM Strain(s)	Tested plant(s)	Effect	Reference
natronolimnaea, *Bacillus* sp.	Wheat (*T. aestivum* L.)		(2017) and Barnawal et al. (2017)
B. subtilis, *B. cereus*, *Rhizobium* spp.	Poplar (*Populus* sp.), Mung bean (*V. radiata* L.)	Soil fertility	Jang et al. (2017), Islam et al. (2016) and Ahmad et al. (2011)
Ochrobactrum sp., *Bacillus* spp., *Pseudomonas* spp., *P. fluorescens*, *B. cereus*, *A. feacalis RZS2*, *P. aeruginosa RZS3*, *Enterobacter* sp. *RZS5*	Rice (*O. sativa* L.), Groundnut (*A. hypogaea*), Maize (*Z. mays* L.), Ashwagandha (*W. somnifera*)	Bioremediation	Pandey et al. (2013), Khan and Bano (2016), Das and Kumar (2016), Kalam et al. (2017a, b), Patel et al. (2016) and Sayyed et al. (2015)
B. subtilis, *A. chroococcum*, *P. putida*, *B. pumilus*, *Exiguobacterium oxidotolerans*	Basil (*Ocimum basilicum*), Brahmi (*B. monnieri* L.)	Secondary metabolites	Banchio et al. (2009) and Ordookhani et al. (2011)

4.1 Characteristics of the Excellent PGPM

If a rhizobacterial strain has certain characteristics that encourage plant development and has the ability to increase plant growth after inoculation, it is regarded as a putative PGPM. (Vejan et al. 2016). PGPM must be very eco-friendly and rhizosphere-competent. After being injected, it ought to significantly multiply and colonize the plant roots. PGPM ought to be capable of encouraging plant development. A wide variety of actions ought to be displayed by PGPM. With other microorganisms in the rhizosphere, it ought to get along. Temperature, desiccation, radiation, and oxidants are examples of physicochemical variables that it should be resistant to.

Compared to the current rhizobacterial communities, PGPM ought to show more competitive abilities.

4.2 Mode of PGPM Action

PGPMs are the predominant microbial population in the rhizosphere and play a role in either actively or passively promoting plant development. They can act as biofertilizers, promoting plant growth and development by strengthening the host plants' resistance to biotic and abiotic stresses and feeding (Sagar et al. 2020; Kalam et al. 2017a, b; Etesami and Maheshwari 2018; Mahdi et al. 2020). These beneficial bacterial groups protect plants and help to stimulate their growth through a number of processes, including root colonization, biofertilization that improves plant physiology and development, induced system-wide resistance, biological control of pathogenic organisms, etc. A detailed examination has been directed of the particular mechanisms by which PGPMs function and how they specifically contribute to the encouragement of plant development (Backer et al. 2018; Dutta and Podile 2010; Khoshru et al. 2020; Ahemad and Kibret 2014; Parray et al. 2016; Swarnalakshmi et al. 2020; Goswami et al. 2016; Khatoon et al. 2020; Oleńska et al. 2020). Traditionally, PGPMs have been thought to work through direct and indirect procedures that occur within and outside of the plant, respectively, to stimulate plant development (Goswami et al. 2016; Glick 2012).

Direct PGPM action can be achieved in two ways: either by controlling the degrees of phytohormones (such as ethylene, auxins, cytokinins, gibberellins, abscisic acid, and others) or by providing phytonutrients that like nitrogen that remain fixed or dispersed minerals from the environment (like P, K, Zn, Fe, and other necessary nutrients) (Parray et al. 2016; Kalam et al. 2020; Gouda et al. 2018). In account to competing with other harmful microorganisms for nutrients and rhizosphere niches, in

conjunction with producing lytic enzymes like chitinases, glucanases, and proteases and antagonistic substances like hydrogen cyanide, siderophores, antibiotics, and antimicrobial metabolites, PGPMs also exert indirect impacts on plant health by preventing phytopathogens and other harmful microorganisms through parasitism. Furthermore, PGPMs cause plants to develop systemic resistance against a range of root and foliar pathogens (Sayyed et al. 2019; Berg et al. 2017). Apart from seeking resources and rhizosphere niches against other detrimental microorganisms, PGPMs also suppress phytopathogens and other harmful microorganisms via parasitism, generating lytic enzymes (like chitinases, glucanases, and proteases) and antagonistic substances (similar to hydrogen cyanide, siderophores, antibiotics, and anti-microbial compounds), and causing systemic resistance in plants against a range of root and foliar pathogens (García-Fraile et al. 2015; Gouda et al. 2018; Vacheron et al. 2013).

4.3 PGPM and Microgreens

The minimal equipment and supply needs, together with a short growth period that ranges from a few days for sprouts to around 2 weeks for microgreens, allow for the quick, easy, and economical production of both microgreens and sprouts. This suggests that there might be a rare chance for industrial scalability additionally to the possibility that people may independently get foods that has demonstrated have nutritional advantages. As a consequence of the enzyme-mediated decomposition of large macromolecules, both sprouts and microgreens are known to be rich in amino acids, fatty acids, and simple sugars in addition to bioactive compounds, specifically a group of secondary metabolites known as phytochemicals, and to contain low levels of antinutrients when compared with seeds or mature plants (Ghoora et al. 2020). PGPMs have gained considerable attention in agriculture due to

their capability to enhance plant growth and productivity. When it comes to microgreens, these beneficial bacteria can have a noteworthyinfluence on various aspects of their growth and development are as follows.

4.3.1 Enhanced Disease Resistance

Some PGPM exhibit intense actions directed against plant diseases (Khan et al. 2022), either by directly inhibiting their growth or by encouraging systemic resistance in plants (Beneduzi et al. 2012). These bacteria can help shield microgreens against a variety of illnesses, which lessens the need for chemical pesticides. PGPMserve as microbial allies in the quest for sustainable and resilient agriculture. Among their myriad benefits, PGPM are valued for their capacity to enhance disease resistance in various crops (Van Esse et al. 2020; Mahmood and White 2019), including the delicate yet potent microgreens.

In the realm of microgreen cultivation, where vulnerability to diseases looms large due to the dense planting, high humidity, and nutrient-rich growth medium, the role of PGPM becomes increasingly crucial. By using a variety of strategies, PGPM directly prevents pathogens (Ahmed et al. 2021) from growing and multiplying, halting their malicious activities before they have a chance to do irreversible damage to the fragile microgreen shoots. One of the primary strategies employed by PGPM is the secretion of antimicrobial compounds (Nath et al. 2023) that act as natural antibiotics, inhibiting the growth of pathogenic bacteria, fungi, and other microorganisms. These bioactive substances, which include antibiotics, enzymes, and secondary metabolites, create a hostile environment for pathogens, disrupting their cellular processes and impeding their ability to colonize and infect microgreens.

By fortifying the microgreen (Guardiola-Márquez et al. 2023) ecosystem with these microbial sentinels, growers

can curtail the incidence and severity of diseases, safeguarding their precious crops from devastating losses. Furthermore, PGPM possess the remarkable ability to induce systemic resistance in plants (Compant et al. 2005; Samaras et al. 2021; Chowdappa et al. 2013), empowering microgreens to mount a formidable defence against invading pathogens. Through intricate signaling pathways and molecular cross-talk, these beneficial bacteria prime the innate immune system of microgreens, activating a cascade of defense responses that fortify their resistance to diseases. This priming effect, akin to vaccinating plants against future infections, enables microgreens to mount a swift and effective counterattack when confronted with pathogenic threats, thus conferring long-lasting protection against diseases.

Moreover, PGPB play a pivotal role in modulating the plant microbiome, shaping the complex interplay between beneficial and pathogenic microorganisms inhabiting the rhizosphere and phyllosphere of microgreens. By outcompeting pathogenic microbes for space and resources, PGPB limit their proliferation and suppress their virulence, thereby reducing the likelihood of disease outbreaks. Additionally, PGPM foster symbiotic relationships with microgreens, forming intimate associations that bolster the plant's resilience to stressors and diseases. Through mutualistic interactions, PGPM enhance the plant's capacity to withstand environmental challenges, bolstering its immune defenses and promoting overall health and vigor.

In addition to their direct antagonistic effects on pathogens, PGPM exert a profound influence on the physiological and biochemical processes underlying disease resistance in microgreens. Through the making of signaling molecules and phytohormones such as salicylic acid, jasmonic acid, and ethylene, PGPM orchestrate intricate defense pathways that fine-tune the plant's response to

pathogens. These signaling molecules serve as molecular messengers, relaying information about impending threats and coordinating the activation of defense genes that fortify the plant's immune system (Bukhat et al. 2020). By modulating these signaling networks, PGPM enhance the plant's capacity to detect and neutralize pathogens, enabling microgreens to mount a robust and coordinated defense against diseases.

Moreover, PGPM enhance the nutritional status of microgreens, bolstering their immune defenses and resilience to diseases. By promoting nutrient uptake and assimilation, PGPM ensure that microgreens receive the essential nutrients needed to support their metabolic processes and synthesis of defense compounds. Additionally, the generation of secondary metabolites having antibacterial qualities is boosted by PGPMsuch as phenolics, flavonoids, and alkaloids, which fortify the plant's immune system and deter pathogenic invaders (Patel et al. 2020; Cappellari et al. 2013). Through these nutritional and metabolic enhancements, PGPM confer a competitive advantage to microgreens, enabling them to resist diseases and thrive in the face of adversity.

4.3.2 Improved Nutrient Uptake

Plants not only require different nutrients, but they also need roots that can uptake nutrients, and PGPM can have a noteworthy influence on the nutrient absorptions. PGPM influence the acquisition of three key macronutrients: nitrogen, phosphorus and iron. Nitrogen is the most important element to plants but cannot be directly accessed from air. For example, diazotrophic PGPM such as *Azospirillum* and *Bacillus* can supply the plant with nitrogen in a more effective form that it can utilize, thereby boosting yield. Similarly, to nitrogen, phosphorus is a crucial nutrient as well. Certain PGPM, for example

Pseudomonas, are capable of a process known as phosphate solubilization, which makes phosphorus more available for plants. This plan has been done and used successfully in switchgrass and tomatoes crop. Iron is an element required for the synthesis of chlorophyll and the general wellbeing of a plant. Similar to PGPM such as *Arthrobacter* and *Trichoderma*harzianum, siderophores, iron-chelating molecules which promote iron assimilation both by plant and beneficial bacteria, are also secreted. This result gives us a glimpse on how PGPM can serve as a sustainable pathway for improving crop produce through enhanced nutrient uptake hence possibly decreasing dependence on chemical fertilizers (Stegelmeier et al. 2022). By solubilizing minerals such as phosphorus and potassium, these bacteria make essential nutrients more accessible to microgreens, leading to improved growth and development.

4.3.3 Stress Tolerance

Microgreens are often grown in regulated settings where they could be exposed to various stresses such as temperature fluctuations, salinity, or drought. ACCD enzyme which plays asignificant role in creation of stress tolerance to plants against biotic as well as abiotic stressors conditions is as such beneficial to their growth under adverse environmental conditions. A comprehensive investigation of the encoding AcdS for ACC deaminase shows that its manifestation exists in various microbes ranging from bacteria to fungus. The plant growth-promoting bacteria together with ACCD gene are advantageous to the plants as they reduce the stress ethylene by breaking down a precursor of ethylene known as 1-aminocyclopropane-1-carboxylic acid (ACC) and generate the cells use ACC as either a source of carbon or nitrogen. A lowered synthesis of ethylene of plants

consequently unbinds the plant growth and development from the ethylene inhibition, additionally, helps plants to avoid different stresses (Singh et al. 2022). PGPM can help mitigate the effects of these stresses by producing stress-relieving compounds like osmo-protectants and phytohormones. This enables microgreens to better withstand adverse environmental conditions and maintain optimal growth.

4.3.4 Promotion of Root Growth

The underground root system of the plant is essential to its aboveground development. The rhizosphere is the region where plant roots and soil microbial communities continuously interact. Through the secretion of root exudates, plants attract rhizosphere bacteria to colonize their internal tissues and root surface. PGPM are a group of microbes that encourage the development of plants through a variety of direct and indirect mechanisms, including as nutrient solubilization, disease control, and biological nitrogen fixation. Many PGPM, by producing phytohormones, volatile organic compounds, and secondary (Benedetto et al. 2017) metabolites play vital role in influencing the root architecture and growth, consequential in increased surface area for the exchange of nutrient and various other rhizosphere effects. By enhancing the root system's physiological functioning, PGPM also increases the root system's efficiency in using its resources. Agroecosystems can benefit from PGPR-mediated root trait modifications by having better crop stands, efficient resource utilization, stress tolerance, soil structure, etc. (Grover et al. 2021). Thus, PGPM capable of harmonizing root traits can play essential function in sustainability in agriculture and root characteristics serve as the main criterion for choosing possible PGPM strains.

4.3.5 Biofertilization

Biofertilizer is becoming a pivotal aspect of organic farming and a significantplayer for the economy and for general agricultural production at a global scale (Bakker et al. 2013). Arbuscular mycorrhizal fungi (AMF), PGPM, and nitrogen-fixing rhizobia are the three main kinds of microorganisms that make up the PGPM and are supposed to be advantageous to plant's growth and nutrition. However, it has been reported that PGPM are used as biofertilizers worldwide, enhancing agricultural productivity and soil fertility. According to earlier research, a biofertilizer made by mixing composts and PGPM may improve the growth-promoting and bio controllable properties of plants. There are two PGPM that have demonstrated efficient bio-control agents are *Bacillus* species and Pseudomonas species. Because PGPR, particularly Bacillus and *Pseuodomonas* species, can develop endospores and withstand a wide variety of environmental conditions, this property makes it easier to formulate biofertilizer. Adequate densities of PGPM in biofertilizer cater a helpful role in creating appropriate rhizosphere for plant's growth and transforming nutritionally important elements through biological process, for example increasing the abundance of N, P, K, besidesinhibiting pathogen growth. Soil can be made fertile by ensuring that there is high N, P, and K availability. In terms of soil fertility, N, P, and K are necessary. Stronger bio-control effects from antagonistic isolates as well as accelerated microorganisms' survival rates in the soil result from the above increasing. By acting as food and enrichment sources for plant, PGPMs act as biofertilizers by helping restore or rebuild the nutrient cycle in the soil, plant roots, as well as among other microorganisms. High N, P, and K availability may increase soil fertility, strengthen the bio-control effects of antagonistic isolates, and increase the rates at which microorganisms survive in

soil. PGPM are considered biofertilizers when they serve as a source of plant feeding and enrichment, restoring or rebuilding the nutrient cycle including the soil, plant roots, and existing microorganisms.

4.3.6 Increased Yield and Quality

All the mentioned factors are the key factors to the production of high yield and quality microgreens. Microgreens effects from raising with PGPM generally are increased number of biomasses, significant improvement of nutritive values, and enhanced total flavour of theirs in comparison with those untreated ones. This characteristic makes them more marketable than the old-fashioned methods and, therefore, yields higher profits for growers. Through PGPM application there were remarkable improvements of the plant weight, number, area, and dry substance of leaves, chlorophyll content and yield and soil nutrients uptake in lettuce grown in soil less condition. Combining 80% mineral fertilizers with PGPR (Ikiz et al. 2024) establisheda lettuce yield that did not majorly differ from the control treatment with 100% mineral fertilizers. Furthermore, the changing in mineral levels and good-for-human-nutrition quality with high phenols, flavonoids, vitamin C, and total soluble solids was detected in the samples of PGPM-treated plants. In addition to being sustainable, the application of PGPM offers the best substitute for mineral synthetic fertilizers in floating hydroponic cultivation for lettuce which, therefore, ensures environmentally friendly and nutritionally advantageous farming. They can have enough nutrients that the plants get enough to grow and make returns. Moreover, they not only reduce chemical synthesis fertilizer dependence but rather it directly enhances plant by providing macronutrients and micronutrient and plant growth hormone promoting. Apart from this, the biopesticides elevate the product quality, boosting crop stress tolerance,

enhance micro biome of soil, and safeguarding against pathogens as well.

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5 Conclusion

The bio-stimulating role of PGPMs is increasingly recognized as a valuable component of maintainable agriculture, including the making of microgreens. PGPMs encompass a diverse array of beneficial bacteria, fungi, and other microorganisms that colonize the rhizosphere and endorse plant's growth through various mechanisms. In the context of microgreens cultivation, harnessing the bio-stimulating properties of PGPMs offers several potential benefits. Firstly, PGPMs can enhance nutrient uptake and assimilation by microgreens through processes such as nitrogen fixation, phosphorus solubilization, and mineralization of organic matter. By making essential nutrients more available to the plants, PGPMs contribute to healthier growth and higher nutritional content in microgreens. Furthermore, PGPMs can stimulate root development and improve overall plant vigour, leading to faster growth and higher yields. This is particularly beneficial in microgreens production, where rapid growth and efficient resource utilization are essential for maximizing productivity. Additionally, PGPMs can enhance the resilience of microgreens to environmental stresses such as drought, salinity, and disease. Some PGPMs produce antimicrobial compounds that inhibit the growth of pathogens, thereby reducing the need for chemical pesticides and fungicides in microgreens cultivation. This not only promotes environmental sustainability but also ensures food safety and quality. Moreover, PGPMs can contribute to soil health and fertility by promoting organic matter decomposition and improving soil structure. Healthy soil ecosystems support beneficial microbial communities, further enhancing the bio-stimulating effects of PGPMs on

microgreens growth and productivity. Incorporating PGPMs into microgreens production can be achieved through various strategies, including inoculation of seeds or growing media with microbial inoculants, application of compost or biofertilizers enriched with beneficial microorganisms, and crop rotation practices that promote microbial variety in soil. Overall, harnessing the bio-stimulating role of PGPMs represents a promising approach to sustainable microgreens production. By fostering symbiotic relationships between plants and beneficial microorganisms, growers can enhance productivity, reduce environmental impacts, and promote the nutritional quality of microgreens, contributing to a more resilient and sustainable food system.

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Recent Advancements in Plant Growth Promoting Rhizobacteria (PGPR) Induced Seed Germination and Seedling Growth and Its Implementation in Soil-Less Leguminous Microgreen Farming

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Keywords Bio-priming – PGPR – Leguminous plants – Seedling growth – Soilless microgreen farming – Seed

Abbreviations

ACC deaminase (1-aminocyclopropane-1-carboxylate) deaminase

AMF arbuscular mycorrhizal fungi

CEA controlled-environment agriculture

EPS exopolysaccharides

IAA Indole-3-acetic acid

NFT nutrient film technique

PGPR Plant growth-promoting rhizobium

ROS Reactive oxygen species

1 Introduction

Microgreens are a modern agricultural innovation that is gaining popularity due to their young, tender shoots that are packed with flavor and nutrition. These crops are grown indoors on a small scale, showcasing the latest practices of controlled environment agriculture. This method is essential in addressing the food needs of urban populations. Microgreens are known for their exceptional nutritional content and unique sensory attributes (Zhang et al. 2021). Legume microgreens, in particular, have a significantly higher zinc content compared to raw seeds and sprouted seeds. This highlights their potential as an excellent source of essential minerals (Butkutė et al. 2018).

Soilless cultivation has become the preferred method for growing microgreens due to several advantages such as controlled environments, space efficiency, reduced disease risk, consistent quality, and sustainability (Fussy and Papenbrock 2022). With the increasing demand for fresh and nutritious greens, soilless cultivation techniques are likely to become even more important in meeting consumer needs and addressing the challenges of modern agriculture (Banerjee et al. 2019). However, soilless cultivation also has some limitations that need to be addressed (Pawełek et al. 2022). One significant limitation is the potential for reduced nutrient availability and microbial diversity compared to traditional soil-based farming. Microgreens grown without soil may lack access to essential nutrients and microorganisms, which can potentially affect their growth and nutritional content (Iqbal et al. 2020). Figure 1 depict the overview of microgreen farming in soilless medium with PGPR for enhancing sustainable microgreen production.

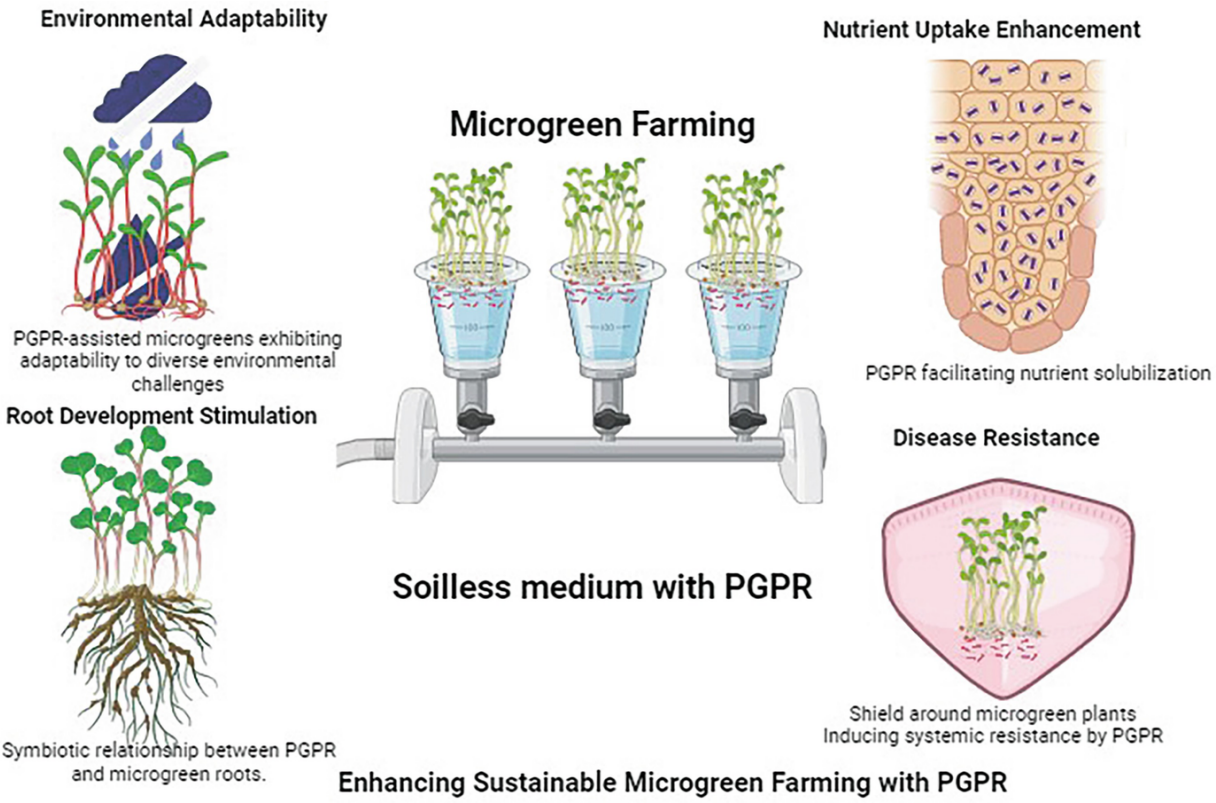


Fig. 1 Overview of microgreen farming in soilless medium with PGPR

Researchers are exploring the use of Plant Growth-Promoting Rhizobacteria (PGPR) to overcome the limitations of soilless microgreen cultivation. PGPR are beneficial bacteria that enhance nutrient uptake, protect against pathogens, and improve stress tolerance (Basu et al. 2021). By adding PGPR to soilless growing media, growers can improve the overall health and nutrient availability of microgreens, leading to better yields and quality (Morales-Cedeño et al. 2021). Additionally, using PGPR can reduce the reliance on synthetic fertilizers and pesticides, making the growing system more environmentally friendly and sustainable (Marteau-Bazouni et al. 2024). By harnessing the natural abilities of these beneficial bacteria, growers can create a more environmentally friendly and sustainable growing system for microgreens (Basu et al. 2021; Grover et al. 2021). PGPR can also suppress soil-borne pathogens by producing antimicrobial compounds and inducing systemic resistance

in plants. (Witkowicz et al. 2020). PGPR also can activate the defense mechanisms of plants, leading to induced systemic resistance (Cao et al. 2023; Zhang et al. 2022). By solubilizing essential nutrients like phosphorus and iron, they make them more accessible to plants (Trivedi et al. 2020). Incorporating PGPR in these practices takes a holistic approach, not only improving cultivation efficiency but also promoting the long-term health and resilience of the entire farming ecosystem (Iqbal et al. 2020).

This chapter provides an in-depth analysis of the significant role PGPR in enhancing the growth of leguminous microgreens. It explores various mechanisms through which PGPR interacts with plants, such as nutrient solubilization, phytohormone modulation, disease suppression, and stress tolerance induction. The chapter also discusses different methods of PGPR inoculation, such as seed bio-priming and soilless farming techniques like hydroponics and aeroponics. Furthermore, it emphasizes the practical implications of PGPR application in leguminous microgreen production, including improved nutrient efficiency, disease resistance, and sustainability.

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2 Mode of Action of PGPR

Leguminous plants are known for hosting rhizobia, which are endosymbiotic root-nodulating bacteria that live in their roots and are capable of capturing nitrogen from the air. Rhizobia encompasses *Azorhizobium, Allorhizobium, Bradyrhizobium, Mesorhizobium, Rhizobium,* and *Sinorhizobium* bacterial genuses. Other than nodule forming rhizobia, several bacterial species like *Agrobacterium, Arthrobacter, Caulobacter, Chromobacterium Erwinia, Flavobacterium, Micrococcus, xylosoxidans, Pseudomonas, Bacillus, Azospirillum, Enterobacter, Azotobacter, Burkholderia, Pantoea, Serratia,*

Acinetobacter, Acetobacter, Azoarcus, Beijerinckia, Herbaspirillum, and *Gluconacetobacter* are identified in rhizosphere of the plant root (Kahindi et al. 1997; Vejan et al. 2016; Swarnalakshmi et al. 2020). These bacteria are called plant growth-promoting rhizobacteria (PGPR) due to their ability to promote plant growth and overall health (Basu et al. 2021). PGPR can be divided into two types: extracellular PGPR (ePGPR), which live on the root surface, and intracellular PGPR (iPGPR), which reside inside the root tissue and nodules. PGPR and plants have a mutually beneficial relationship, as PGPR can enhance plant growth in a variety of ways. The use of PGPR is a promising approach for sustainable agriculture (Grover et al. 2021). Plant Growth Promoting Rhizobacteria (PGPRs) are known to possess several beneficial traits such as atmospheric nitrogen fixation, phosphate solubilization, regulation of phytohormone levels, production of volatile compounds, and anti-pathogen defense. PGPRs can solubilize essential nutrients like phosphorus and iron, which in turn makes them more accessible to plants (Trivedi et al. 2020). As a result, improved nutrient uptake and utilization fosters healthier plant development. Additionally, PGPRs have the potential to suppress soil-borne pathogens through the production of antimicrobial compounds and the induction of systemic resistance in plants (Cao et al. 2023). Many PGPR strains produce phytohormones like auxins, cytokinins, and gibberellins, which play crucial roles in regulating plant growth and development. Moreover, PGPRs have been found to enhance specific phytochemical and secondary metabolite production, thus improving the quality and value of agricultural products (Khalediyan et al. 2021). Certain PGPR strains also enhance plant tolerance to drought and other environmental stresses. They can produce osmoprotectants and facilitate water absorption, helping plants cope with challenging conditions and ensuring sustained growth (Zhang et al. 2022).

PGPR have a positive impact on plant growth in both optimal and suboptimal environmental conditions, and enhance plant tolerance towards biotic and abiotic stress. Through their interaction with plants, PGPR have evolved to accumulate genes that are necessary for plant growth and tolerance. In the rhizosphere, which is the soil surrounding plant roots, plant-microbe interactions are majorly mediated by the root exudates that plants release (Bukhat et al. 2020). The composition of these exudates plays a significant role in altering microbial diversity in the rhizosphere consortium. Root exudation provides energy and regulates metabolic activities of the PGPR that are associated with the plant. Furthermore, the composition of root exudation is influenced by environmental factors like temperature, salinity, pH, etc., and varies from plant species to species. This variability selectively favors the growth of specific microbiota that are unique to each plant species and are adapted to its specific environment (Garrido-Oter et al. 2018).

Agricultural innovations have traditionally focused on the use of synthetic fertilizers and pesticides, which can have long-lasting negative impacts on the environment and ecosystems. To address this issue, researchers have been exploring the use of PGPR to enhance agricultural yield while minimizing harm to the environment.. There are several ways to effectively inoculate plants with PGPR, either by using a single PGPR species or a combination of different strains. The most common methods involve inoculating seeds, roots, or the soil (Lopes et al. 2021). Seed bio-priming involves submerging seeds in a solution containing PGPR, while roots can be incubated with PGPR solution to introduce the microorganisms into the root microbiota (Mahmood et al. 2016). Interestingly, no significant difference in yield was observed for different inoculation methods. This suggests that all of these methods can be effective ways to inoculate plants with

PGPR and achieve enhanced agricultural yield while minimizing harm to the environment. (Hernández-Montiel et al. 2017).

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3 Effect of PGPR on Seed Germination

The quantity of microgreens produced can vary depending on how well the seeds germinate. Several factors can affect the efficiency of seed germination, such as seed dormancy, size, maturity, coat permeability and genetics. Every seed has a different germination ability, making seed germination a non-uniform process. This variability can negatively impact the quality and yield of the microgreens. Additionally, germinating seeds and seedlings are more sensitive to environmental stresses than mature plants. Environmental stresses like temperature, pH, and osmolality can significantly affect seed germination and seedling growth. To improve seed germination efficiency and uniformity, seed bio-priming with PGPR has been reported to be effective (Mahmood et al. 2016). Major agricultural crops such as rice, wheat, and cucumber exhibit notable improvements in seed germination percentage, seed vigor, and uniformity when treated with PGPR, both in normal and stressful conditions (Prasad et al. 2017; Sarkar et al. 2018; Pérez-García et al. 2023). Recent studies on legume seed bio-priming have reported significant enhancements in seed germination efficiency and extended effects on improved seedling growth. PGPR stimulates seed germination by modulating the production of phytohormones like gibberellins and IAA. Abscisic acid induces and maintains seed dormancy while negatively regulating germination. Gibberellins and IAA are essential for germination and counteract the effect of abscisic acid (Miransari and Smith 2014; Hussain et al. 2020). The

production of gibberellins increases in mustard seed when incubated with PGPR compared to the control. Moreover, PGPR such as *Bacillus casamancensis* strain MKS-6 and *Bacillus sp*. Strain MRD-17 have been found to enhance mustard seed germination under osmotic stress. (Nivetha et al. 2024). Additionally, *B. japonicum, A. brasiliense*, and *P. rhizosphaerae* were reported to enhance seed germination efficiency and nodule formation in soybeans by increasing the production of gibberellins and IAA (Cassán et al. 2009). Soybean seed bio-primed with *Bacillus megaterium* also showed improved germination and seedling growth (Miljaković et al. 2022). A recent study on chickpeas found that seed bio-priming with a consortia of *Bacillus Pseudomonas* and *Mesorhizobium* PGPR species improved germination percentage up to 93% (Swetha and Dayal 2024). Bio-priming lentil seed with *Azotobacter* improved germination percentage up to 88.66%. (Kumar et al. 2016). Furthermore, co-inoculation of Rhizobium and *Pseudomonas* by seed bio-priming significantly improved chickpea seed germination (Vishwas et al. 2017). *Trichoderma viride, Trichoderma harzianum,* and *Pseudomonas fluorescence*-mediated bio-priming were identified to improve germination percentage and germination rate in common beans (*Phaseolus vulgaris* L.) (Monalisa et al. 2017). In addition to live microorganisms, the signaling molecule Thuricin17 produced by *Bacillus thuringiensis* alone also has a positive impact on seed germination and seedling growth in canola (*Brassica napus* L.) (Nazari et al. 2022) (Table 1).

Table 1 Effect of various PGPRs on plant and seedling growth of legumes

Legumes Type	PGPR strain	Effect on Plant growth, seedling and germination			References
		Shoot	Root	Biomass	
Mung bean *(Vigna radiata)*	Pseudomonas sp. PS1	Total chlorophyll content and, and shoots	Increase in nodule counts, phosphorus content and leghaemoglobin, nitrogen in the roots	Increase in plant dry weigh	Hussain, Sabir (2020)
Soybean (*Glycine max* L.)	*Pseudomonas* sp.	Enzyme activity increase	Soil productivity, and nitrogen absorption of root increased	–	Kumawat et al. (2022)
Common pea (*Pisum sativum*)	*Rhizobium strain* MRP1	Protein content of pea plants increased.	Increse nodulation and hemoglobin content	–	Dasgupta et al. (2023)
Mung bean *(Vigna radiata* L.)	*Rhizobium phaseoli*	Increased plant height,	Nodule density increase	Biomass increase	Dhole et al. (2023)
Chickpea (*Cicer arietinum*)	*Bacillus* PSB10 SP.	Increased chlorophyll content	Increased nodulation and leghemoglobin	–	Verma et al. (2019)
Chickpea (*Cicer arietinum*)	*Pseudomonas* sp.	–	–	Increased biomass	Lyu et al. (2022)

4 Effect of PGPR on Seedling Growth in Optimum Environmental Conditions

Under optimal conditions, seedlings that are inoculated with PGPR display significantly improved growth compared to un-inoculated control groups. Studies have shown that PGPR-inoculated seedlings of common agricultural crops such as rice, wheat, and maize exhibit improved root and

shoot growth parameters as compared to the control sets. Recent research has revealed that PGPR inoculation, particularly with *Trichoderma spp*. and *Bacillus subtilis* through seed bio-priming, enhances the growth of bean seedlings (Junges et al. 2016). Chickpeas have also shown improvement in seedling growth and biomass when seeds are bio-primed with a consortium of *Bacillus Pseudomonas* and *Mesorhizobium* species (Swetha and Dayal 2024). Likewise, PGPR-mediated seed treatment has been reported to have similar effects on French beans and lentils (Kumar et al. 2016; Negi et al. 2019).

PGPR not only enhance root and shoot development in seedlings, but they also increase nitrogen and phosphorus uptake and the content of photosynthetic pigments in plants. Complete genome analysis of different PGPR strains has identified the presence of one or more genes involved in nutrient assimilation (Bruto et al. 2014). PGPR strains involved in nitrogen fixation and improving ammonium assimilation often possess nitrogenase genes (nifHDK). Phosphate is the second most growth-limiting nutrient due to its poor solubility over a wide range of pH. PGPR produce organic acids like citric acid, formic acid, and most importantly, gluconic acid to solubilize phosphate from inorganic salts like $Ca_3(PO_4)_2$ (Miller et al. 2010). Gluconic acid is produced by pyrroloquinoline quinone (pqqE) and glucose dehydrogenase enzymes, which are frequently found in different PGPR strains. Gluconic acid solubilizes phosphate by chelating the metal counterpart of insoluble phosphate salts. Several other enzymes produced by PGPR like acid phosphatase (olpA), alkaline phosphatase (phoD), phytase (appA), phosphonatase (phnX), and C-P lyase (phnJ) have phosphate solubilizing abilities (Alori et al. 2017; Liang et al. 2020). For legume plants like common bean (*Phaseolus vulgaris* L.), inoculation of free-leaving phosphate solubilizing PGPR like *Paenibacillus polymyxa*

and *Bacillus megaterium* shows a synergistic effect on growth improvement in addition to symbiotic rhizobia (Korir et al. 2017). Availability of phosphate initiates nodule formation, therefore, co-inoculation of phosphate solubilizing PGPR with rhizobia was reported to synergistically improve nodulation, N_2 fixation and overall plant growth (Olivera et al. 2004; Korir et al. 2017). The availability of soluble iron (Fe^{2+}), an essential micronutrient, affects the overall growth and disease-preventive ability of plants. However, in an aerobic environment, atmospheric oxygen oxidizes soluble Fe^{2+} to insoluble Fe^{3+}. PGPR are reported to produce siderophores, organic molecules that can chelate Fe^{3+} and solubilize it for easy uptake by the host plant. Siderophore-mediated iron assimilation was reported to reduce reactive oxygen species, prevent phytopathogens and improve growth (Sayyed et al. 2013; Kumar et al. 2024). A recent study identified *Pseudomonas sp.* that secreted pyoverdine siderophore and improved iron assimilation in peanuts (Wang et al. 2024).

PGPR, are known to stimulate plant growth by producing plant hormones like auxins, cytokinin, and NO while reducing the level of ethylene. Auxins and NO are linked with nodulation (Pii et al. 2007). Research has shown that PGPR like *B. japonicum* SB-1 and *Bacillus thuringinesis* KR1 enhance early nodulation, efficient N_2-fixation, and overall yield in peas and lentils by promoting enhanced auxins production (Mishra et al. 2009). PGPR also produce indole acetic acid and volatile NO through an indole-3-pyruvate decarboxylase gene and copper nitrite reductase gene (nirK), respectively. Both IAA and volatile NO control plant root structure and enhance lateral root growth, thereby increasing nutrient and water uptake capability of the seedling, which positively affects overall growth (Li et al. 2021). Recent studies have shown that *Pseudomonas* sp.

CM11 prevents primary root growth while enhancing lateral root formation, shoot, and biomass growth in Arabidopsis seedlings (Li et al. 2022). PGPR strain *Phyllobacterium brassicacearum* was found to enhance 50% lateral root growth in Arabidopsis seedlings, and this ability is directly correlated to the auxins production capacity of the PGPR (Contesto et al. 2010). *Azospirillum brasilense* Sp245 was also found to enhance lateral root growth in tomato seedlings by stimulating NO synthesis (Creus et al. 2005).

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5 Effect of PGPR on Seedling Growth Under Stress Conditions

Abiotic stresses, such as temperature fluctuations, salinity, and drought, can pose significant risks to agricultural plant growth and yield. These stresses are difficult to control in open-field settings and even in controlled greenhouse conditions. In soilless indoor farming systems, abiotic stress can significantly reduce overall yield. PGPR can help to reduce the growth-retarding effects of abiotic stress by activating stress response systems already present in plants through the regulation of gene expression. Moreover, PGPRs themselves produce and secrete various biochemical compounds, such as exopolysaccharides (EPS), non-enzyme antioxidants, indole-3-acetic acid phytohormone, and more. PGPR also increases enzymes like 1-aminocyclopropane-1-carboxylate (ACC) deaminase, antioxidant enzymes like lipid peroxidase, etc. These mechanisms help plants better cope with abiotic stressors, leading to enhanced resilience and productivity even in challenging environmental conditions. Plants release excessive amounts of ethylene, which acts as a stress hormone when they are under stress. Ethylene can hinder growth and even have deadly effects when present at high concentrations. Fortunately, the

majority of PGPR produces the enzyme ACC deaminase, which breaks down excess ACC, a precursor of ethylene hormone produced and secreted by the host plant (Dutta et al. 2022). PGPR breaks down ACC into inactive α-ketobutyrate and uses it as a nitrogen source. By breaking down ACC, PGPR reduces ethylene levels and induces tolerance to abiotic stresses such as temperature fluctuations, salinity, pH changes, etc. Recent studies have shown that using PGPR like *Bacillus tequilensis* SSB07 and *Bacillus cereus* SA1 can improve high-temperature tolerance in soybean seedlings (Kang et al. 2019; Khan et al. 2020). Bacillus subtilis has been found to improve heat stress tolerance in common bean (de Lima et al. 2022). High temperatures can destabilize proteins, deactivate plant enzymes, and hinder metabolic processes. However, the presence of PGPR has been found to induce the overexpression of heat shock proteins (HSP) in plants, which stabilizes metabolically important proteins (Shaffique et al. 2022). On the other hand, cold temperatures slow down metabolic activity and reduce plant growth. PGPR has been reported to increase cold tolerance in wheat seedlings (Mishra et al. 2011) and other plants (Zhang et al. 2023) by reducing electrolyte leakage through damaged membranes. Additionally, PGPR can help prevent the damaging effects of high salinity and heavy metal contamination. For example, *P. putida* UW 4 was reported to improve soot and root biomass growth in canola (*Brassica napus*) seedlings under salinity stress (Cheng et al. 2012). Consortia of *Aneurinibacillus aneurinilyticus* and *Paenibacillus sp.* can also improve seed germination and seedling growth in common beans under salinity stress (Gupta and Pandey 2020). High salinity can cause Na+/K+ imbalance in plant tissue, impair nutrient uptake through membranes, impair germination rate, and plant growth (Ha-Tran et al. 2021) (Fig. 2). However, PGPR produces exopolysaccharides

(EPS) that can help sequester Na+ ions to mitigate salt stress (Khan and Bano 2019). Moreover, PGPR interacts with plants and induces the production of osmolytes like choline, glutamate, glycine betaine, proline, trehalose, etc., which can help overcome drought and salinity stress.

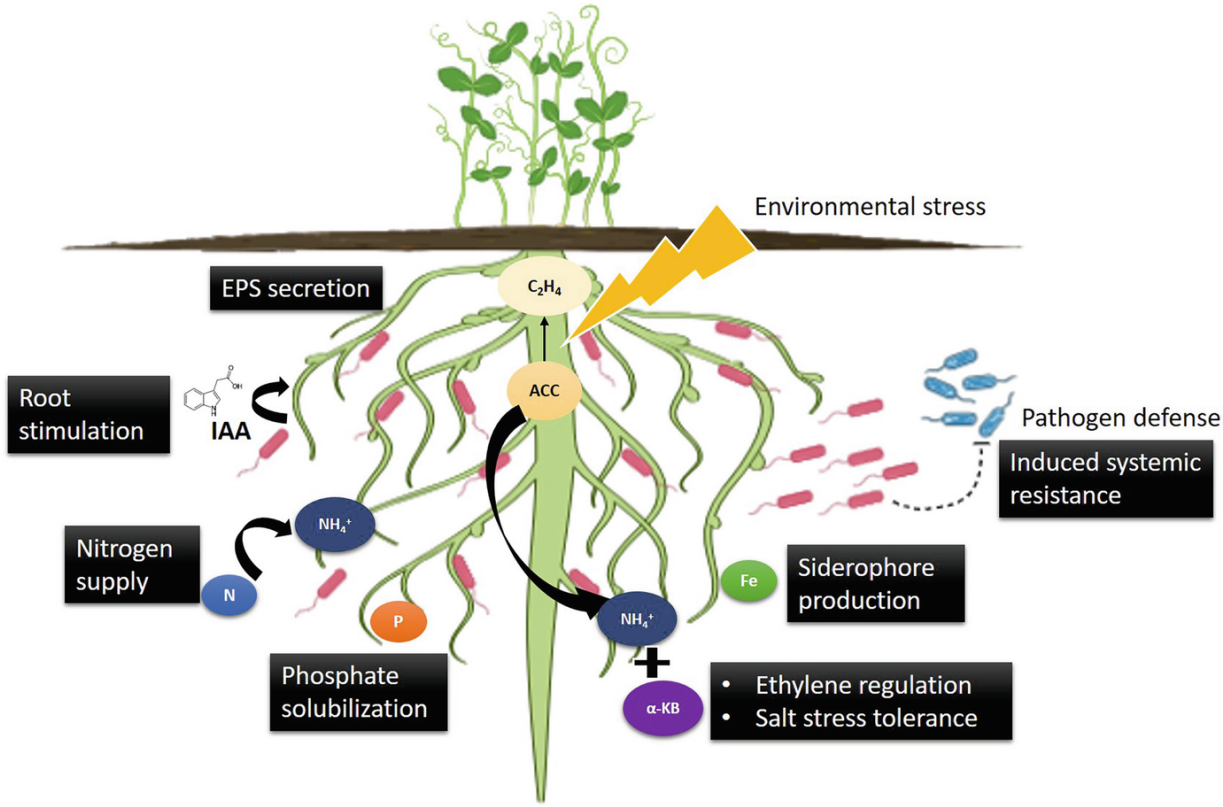


Fig. 2 Effect of PGPR on growth of leguminous plants under stress condition

Biotic stress is a major cause of reduced crop yield in outdoor farming. However, indoor-grown plants are less susceptible to disease. For legumes, fungal pathogens can cause diseases such as rust, powdery mildew, wilt, ascochyta blight, chocolate spot and root rot disease, which can lead to yield reductions of up to 100% (Mahmoud 2021). The use of synthetic pesticides to treat biotic stress can have harmful long-term effects on the environment, farmers and consumers. Recent research on PGPR shows promise in mitigating biotic stress in a sustainable way. Studies have shown that the inoculation of chickpea

seedlings with *Pseudomonas aeruginosa* and *Pseudomonas putida* can prevent fungal infections such as root rot and charcoal rot diseases (Akhtar and Siddiqui 2009; Khare and Arora 2010). PGPR produce chitinase, glucanase, HCN, antifungal compounds, siderophore and other antimicrobial compounds to enhance plant growth and induce biotic stress tolerance (Sharma et al. 2018; Verma et al. 2018). Reactive oxygen species (ROS) can induce stress and have a growth retarding effect, but plants produce H_2O_2 to fight pathogens and improve cell wall structure. PGPRs produce IAA and ACC deaminase to induce overproduction of antioxidant enzymes in plants, helping to remediate stress in chickpeas (Khare and Arora 2010; Singh et al. 2013). In addition to fungal diseases, PGPR are also effective against bacterial diseases. *Bacillus subtilis* has been shown to improve yield by alleviating bacterial wilt disease in common beans (Martins et al. 2013) (Table 2).

Table 2 Plant metabolites (Hormones) secreted by PGPR inoculation which promote growth

Legumes Type	**PGPRs strain**	**Plant metabolites secretion**	**References**
Alfalfa (*Medicago sativa* L.)	*Acinetobacter* spp.	IAA, phosphate solubilization, and siderophores	Kiruthika and Arunkumar (2020), Tafaroji et al. (2022)
Chickpea (*Cicer arietinum* L.)	*Bradyrhizobium* sp.	IAA, ammonia, siderophores, and exo-polysaccharides	Kiruthika and Arunkumar (2020)
Chickpea (*Cicer arietinum L.*)	*Bacilus* spp. PSB10	IAA, ammonia, siderophores	Wani et al. (2007)
Mung bean (*Vigna radiata*)	*Bradyrhizobium japonicum*	IAA	Zveushe et al. (2023)
Glycine max *(*L.*)*	*Bradyrhizobium japonicum*	IAA	

Legumes Type	PGPRs strain	Plant metabolites secretion	References
Chickpea (*Cicer arietinum* L.)	*Enterobacter asburiae*	IAA, siderophores, phosphate solubilization, ammonia, and exo-polysaccharides	Saikia et al. (2023)
Cajanus cajan	*Enterobacter* sp.	Phosphate solubilization and ACC deaminase, IAA, and siderophores	Singh et al. (2022)
Common bean (*Phaseolus vulgaris* L)	*Flavobacterium*	IAA and siderophores	Feng et al. (2023)
Chickpea (*Cicer arietinum* L.)	*Mesorhizobium* sp.	Exo-polysaccharides, IAA, siderophores, ammonia	Yadav et al. (2022)
Chickpea (*Cicer arietinum* L.)	*Pseudomonas aeruginosa* OSG41	Salicylic acid, 2, 3-dihydroxybenzoic acid siderophores, phostphate-solubilization and IAA	Pereira (2019)
Lentil (*Lens culinaris ssp. Culinaris*)	*Rhizobium sp. lentil*	Ammonia, IAA, siderophores	Alinia et al. (2022)
Common bean (*Phaseolus vulgaris* L.)	*Rhizobium phaseoli*	IAA	Maslennikova et al. (2022)
Common bean (*Phaseolus vulgaris* L.)	*Rhizobium leguminoserum* bv phaseoli	IAA, ACC deaminase, and siderophore	Kamran et al. (2017)

6 Soilless farming of Legume Microgreens Using PGPR

The increasing interest in soilless microgreen farming is primarily driven by sustainability concerns. Soilless microgreen farming optimizes resource utilization,

requiring less water and space than traditional soil-based methods (Fussy and Papenbrock 2022). This cultivation approach mitigates the environmental impact associated with conventional agriculture, minimizing soil degradation, reducing the need for chemical inputs, and enabling more efficient waste management. Thus, it contributes to the overall environmental sustainability of agriculture (Banerjee et al. 2019). The integration of technological advancements in agriculture has led to the development of controlled-environment agriculture (CEA). Soilless microgreen farming offers an efficient and controlled method of producing nutrient-rich greens in a short period. As this innovative farming method gains momentum, there is a parallel recognition of the crucial role of PGPR in ensuring its sustainability. Integrating PGPR into soilless microgreen systems can enhance growth, nutrient uptake, and overall plant health. PGPR can be employed in several types of soilless microgreen farming .

6.1 Hydroponic Systems for Legume Microgreen Farming

The hydroponic system is one of the different soilless cultivation methods adopted recently for legume microgreen farming. The major advantages of the hydropony cultivation method include reduced water consumption, less pathogens and pest mediated crop loss and easy to control of nutrient and environmental conditions, potential for year-round production and higher productivity. For hydroponic farming, the use of high-quality legume seeds as well as bio-priming with PGPR enhances germination efficiency. The use of oxygen saturated water in hydroponic systems reported to induce faster germination, rapid growth of root systems and increased productivity of legume microgreens (Grishin et al. 2021). Moreover, legume microgreen farming with modern hydroponic systems reduces production time by

accelerating growth and biomass production as well as positively impacts quality by enriching phytochemical components like specialized metabolites and antioxidants (Fabek Uher et al. 2023). The hydroponic system requires growing medium such as coconut coir, perlite, or rockwool, which can be used as support for the shoots and serves as effective mediums due to their water retention ability and porosity that enhance aeration to the roots (Eswaranpillai et al. 2023). The nutrient solution need to be optimized based on the specific requirements of the legume species to ensure availability of essential nutrients and efficient growth. Furthermore, legume microgreen production in hydroponic system reported to get enhanced by use of LED lights and temperature, humidity control environments (Sharma et al. 2023). Legume microgreens grown in hydroponic system are harvested typically when shoots are around 2–4 inches tall. However, Kyriacou et al. identified that a faster growth rate influenced by modernized hydroponic system can reduce the quality of the legume microgreen by decreasing health promoting phytochemical contents (Kyriacou et al. 2020). Furthermore, brassicaceous species microgreens are found to accumulate harmful nitrate from nutrient media when grown in specific organic medium like peat moss (Kyriacou et al. 2020). Nitrate deprivation by growing brassicaceous microgreens in nitrate-free nutrient media for 2–5 days before harvest may reduce harmful nitrate content (Colla et al. 2018).

Introduction of PGPR into the hydroponic system found to be advantageous with improved nutrient availability, stimulated root growth and suppressed harmful plant pathogens (Antonius et al. 2021). The addition of PGPR in hydroponic systems reported to promote healthier root development and overall plant growth (Dhawi 2023). The PGPR can be introduced into the nutrient solution to enhance nutrient availability as well as reduce the chemical requirements for legume microgreens farming.

Commercially available bacterial biostimulant consortium enhanced biomass growth and salt stress tolerance in green leafy vegetables grown in a hydroponic system (Moncada et al. 2020). In addition, the PGPR found to effectivly prevent root rot and damping-off disease in hydroponic mustard microgreen farming (McGehee et al. 2019). Biofilm forming PGPR are more effective in colonizing the rhizosphere due to stickness of the biofilm and thus have additional advantages in hydroponic system (Sato et al. 2023). Optimitation of the nutrient media composition with the addition of carbohydrates like sucrose or trehalose can induce biofilm production of certain PGPR (Welbaum et al. 2009).

6.2 Aeroponic and Vertical Systems for Legume Microgreen Farming

Aeroponic systems offer a convenient method for cultivating leguminous microgreens and studying plant-microbe interactions, particularly beneficial for phenotyping roots and monitoring nodule development in legumes (Liu et al. 2019). These systems allow precise control of the growth medium and easy root observations, making them ideal for research purposes (Jones-Baumgardt et al. 2020) (Fig. 3). Aeroponic systems suspend microgreen roots in the air and periodically mist them with a nutrient solution. PGPR can be added to the nutrient mist to colonize the root zone, promoting nutrient absorption and improving plant resilience. Aeroponic systems offer efficient nutrient delivery and can benefit from the addition of PGPR to enhance microgreen growth. Additionally, wireless sensor networks can be implemented in aeroponic greenhouses to monitor environmental parameters in real-time, aiding in maintaining optimal growing conditions for leguminous crops (Cai et al. 2023). Furthermore, the use of supplemental lighting, such as LED technology, can enhance the growth and yield of microgreens like kale and

arugula, ensuring consistent production levels year-round even in low light conditions (Méndez-Guzmán et al. 2022). By integrating beneficial plant growth-promoting rhizobacteria into aeroponic systems, researchers can explore the symbiotic relationships between leguminous plants and microbes, potentially enhancing plant growth and nutrient uptake for sustainable agricultural practices.

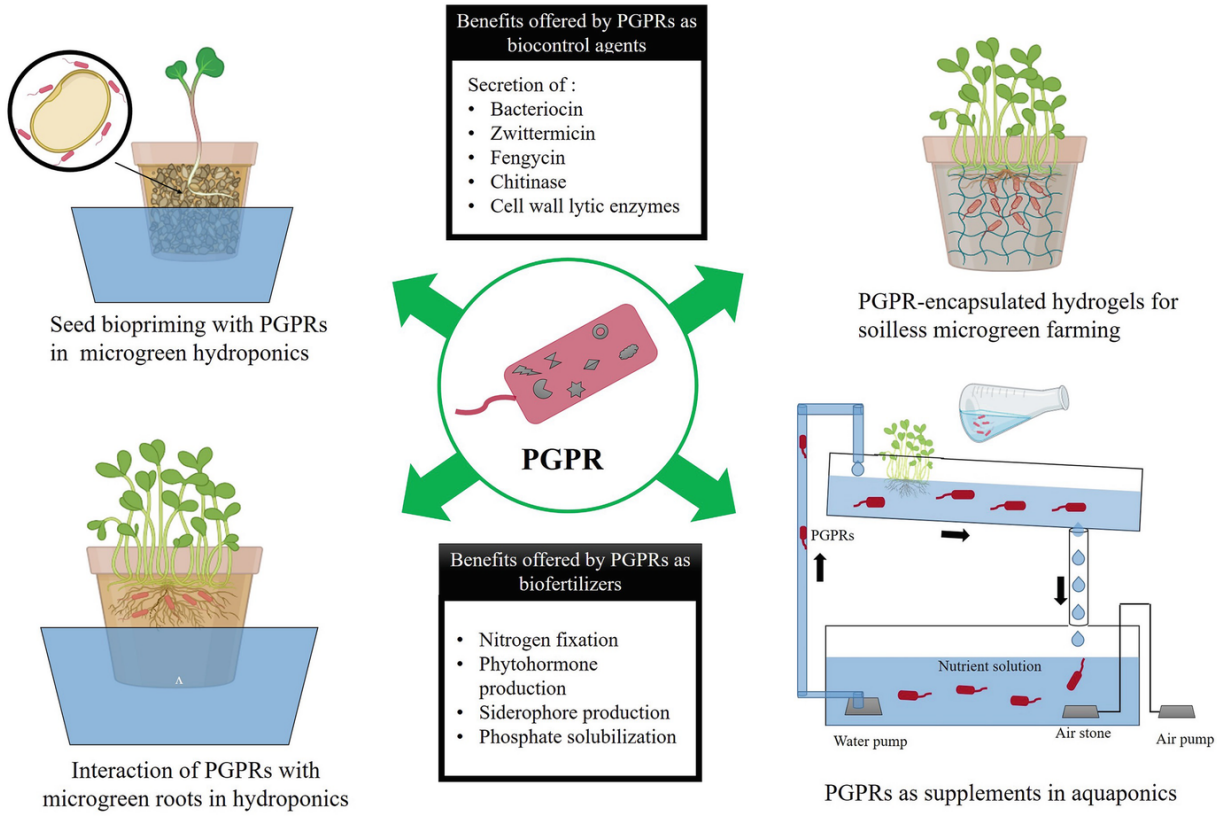


Fig. 3 Application of PGPR in various soilless microgreen farming

Furthermore, Vertical farming, a space-efficient method of cultivating crops in stacked layers indoors, offers a promising opportunity to enhance microgreen production by integrating PGPR into the growing medium or nutrient solution. Research by Wiggins et al. (2020) demonstrated the feasibility of incorporating PGPR into hydroponic systems, leading to improved lettuce productivity. Similarly, Dasgan et al. (2023) found that PGPR and and arbuscular mycorrhizal fungi (AMF) treatment in hydroponic lettuce

cultivation resulted in the highest growth parameters and essential nutrient content, indicating its potential to optimize lettuce growth. Several PGPR have been tested in soil-free vertical farming of strawberries, lettuce, tomato, and *Glycine max* (L.) Merr., with improved growth and nutrient uptake.

6.3 Nutrient Film Technique for Legume Microgreen Farming

A nutrient film technique (NFT) is a method that involves circulating a thin film of nutrient solution over microgreen roots, which allows for efficient nutrient uptake. To improve nutrient availability and promote healthier root systems, PGPR can be introduced into the nutrient solution. NFT systems offer precise control over nutrient delivery and can benefit from PGPR application to optimize microgreen growth. A study reported the use of a porous hydrogel composite that combined an agarose network and porous growing mix particles to improve water retention and root zone aeration for plant cultivation (Teng et al. 2022). The development of a hydrogel composite for red cabbage microgreens, as discussed by Teng et al. (2022), has shown promising results in sustainable growth cycles without the need for watering or crew interaction. By enhancing nutrient delivery through increased total pore volume and larger pores, the hydrogel composite achieved a remarkable 54% increase in microgreen yield compared to a pristine hydrogel. Moreover, this composite demonstrated satisfactory support for microgreen growth under simulated microgravity conditions, highlighting its potential for indoor and space farming applications. This innovative substrate not only offers a lightweight and convenient solution but also proves to be water-efficient, addressing the challenges of agricultural sustainability and resource management. (Fig. 3).

7 Future Perspectives and Conclusion

Plant Growth-Promoting Rhizobacteria (PGPR) play an essential role in enhancing the growth and productivity of plants, especially in leguminous crops. The field of PGPR application in leguminous crop production is evolving with emerging trends and technologies. High-throughput sequencing technologies allow researchers to explore microbial communities in the rhizosphere, providing insights into the dynamics of PGPR-plant interactions. Functional genomics techniques, such as CRISPR-Cas9, can manipulate the genetic makeup of PGPR strains to enhance their beneficial traits. Metagenomics approaches can identify specific genes and metabolic pathways in PGPR that contribute to plant growth promotion. Synthetic biology can design and engineer custom PGPR strains with enhanced capabilities for nutrient solubilization, nitrogen fixation, and disease resistance, tailored to specific leguminous crops and environmental conditions. Omics technologies provide a comprehensive understanding of the molecular mechanisms underlying PGPR-mediated plant growth promotion. The development of novel bio-stimulants and bio-fertilizers based on PGPR strains is gaining attention. These products can improve nutrient uptake, water use efficiency, and overall plant health in leguminous microgreens. Commercial formulations incorporating PGPR are marketed as eco-friendly alternatives to conventional fertilizers. With climate change’s increasing impact on agriculture, there is a growing focus on developing soil-less farming and PGPR strains that can enhance the resilience of leguminous crops to environmental stressors. The development of regulatory frameworks for the commercial use of PGPR products is gaining importance. Clear guidelines and standards ensure the safe and responsible deployment of these technologies in agriculture. The convergence of these trends and technologies is expected

to significantly enhance the efficiency and sustainability of leguminous microgreen production through the application of PGPR. The practical implications of using PGPR in leguminous seedling growth and soilless microgreen farming encompass improved nutrient efficiency, cost reduction, disease resistance, accelerated growth, stress tolerance, optimized root development, customization for specific crops and environments, consistent crop quality, integration with precision agriculture, and compliance with organic standards. These practical benefits make PGPR a valuable tool for modern and sustainable microgreen production.

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Genetic Enhancement of Leguminous Microgreens: A Frontier in Sustainable Nutrition

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Abbreviations

SDGs Sustainable Development Goals
AGRI Ministry of Agriculture and Irrigation, Alberta
PEM Protein Energy Malnutrition
SPD Spermidine
CAD Cadaverine
AGM Agmatine
SPM Spermine
PUT Putrescine
NGS Next-generation Sequencing
ESTs Expressed Sequence Tags
SNPs Single Nucleotide Polymorphisms
QTLs quantitative trait loci

RNA Ribonucleic Acid

DNA Deoxyribonucleic Acid

SSR Simple Sequence Repeats

AFLP Amplified Fragment Length Polymorphism

PSY Phytoene Synthase

PSMs Plant Secondary Metabolites

PGPR Plant Growth-Promoting Rhizobacteria

AM Association mapping

Rashmi Rani and Shagolshem Yaiphabee Chanu contributed equally with all other contributors.

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1 Introduction

The prevalence of chronic diseases is a pressing concern often associated with various factors, including poor diet quality, malnutrition, and aging populations, particularly in urban environments. Urbanization tends to bring about shifts in dietary habits, often leading to an increased consumption of processed foods while also decreasing the intake of fresh fruits, vegetables, and other nutritious foods, thus leading to various lifestyle disorders. Additionally, as populations age, they become more susceptible to chronic conditions such as cardiovascular diseases, diabetes, and certain cancers. These trends underscore the importance of promoting healthy eating habits, improving access to nutritious foods, and implementing lifestyle interventions to mitigate the burden of chronic diseases in urban areas. Urbanization, coupled with an aging population, exacerbates these challenges, while the alarming decline in biodiversity further compounds the issue. The biodiversity factsheet published by the University of Michigan in 2024 highlights a staggering 69% decline in wildlife populations since 1970, emphasizing the critical state of our ecosystems. Meanwhile, the World Bank's report reveals a grim reality: despite substantial budget allocations, millions still face acute food insecurity globally. The interconnectedness of chronic diseases, poor diet quality, and environmental degradation underscores the urgent need for sustainable solutions in food production and nutritional security. Addressing these complex issues demands evidence-based strategies that prioritize

environmental health, resource sufficiency, and nutritional quality. As outlined by Johnson et al. (2020), any viable solution must be both economically feasible and environmentally sustainable. Only through concerted efforts can one hope to safeguard both human health and the planet's future.

Microgreens, often referred as "living superfoods" or "functional foods," offer a promising avenue for addressing the Sustainable Development Goals (SDGs) established by the UN in 2015 to tackle global crises. These miniature wonders are the young, edible seedlings of various plants, including herbs, vegetables, and legumes, harvested at an early growth stage, typically within 1 to 2 weeks. Serving as an intermediary stage between sprouts and mature greens, microgreens boast of a unique blend of rich flavor and aroma (Di Gioia and Santamaria 2015). The distinctions between sprouts, microgreens, and mature plants are depicted in Fig. 1, revealing that there are only a handful of differences between them. Microgreens are slightly older than sprouts but younger than mature plants. They have developed their first set of true leaves and are harvested when they are about 1–3 inches tall. They are larger than sprouts, with the development of true leaves and often more pronounced stem growth. They are also highly nutritious, often containing higher levels of vitamins, minerals, and antioxidants compared to mature plants. They have a more intense flavor compared to sprouts and are used similarly in salads, sandwiches, soups, and as garnishes to add flavor and texture. Their aroma and flavor can be attributed to an impressive array of phytoactive compounds, including vitamins, minerals, antioxidants, and phytochemicals. Research indicates they are rich sources of essential nutrients such as vitamin C, vitamin B complex, folate, and minerals like phosphorus, manganese, zinc, iron, copper, calcium, magnesium, selenium and potassium. Moreover, microgreens contain compounds like sulforaphane, chlorophyll, flavonoids, polyphenols, lignans, coumetarols, phytosterols, and glucosinolates, antioxidants and carotenoids, known for their health-promoting properties. They are also rich in triterpenes, saponins, and condensed tannins (Butkutė et al. 2018; Sharma et al. 2023). With their dense nutritional profile and quick cultivation cycle, microgreens present a sustainable and accessible means of enhancing dietary diversity, promoting health, and contributing to the achievement of the SDGs. Microgreens of families like Fabaceae, Apiaceae, Asteraceae, Amaranthaceae, and Brassicaceae

exhibit antimicrobial, anti-inflammatory, antioxidant, and anti-carcinogenic properties, contributing to various health benefits (Gupta et al. 2023).

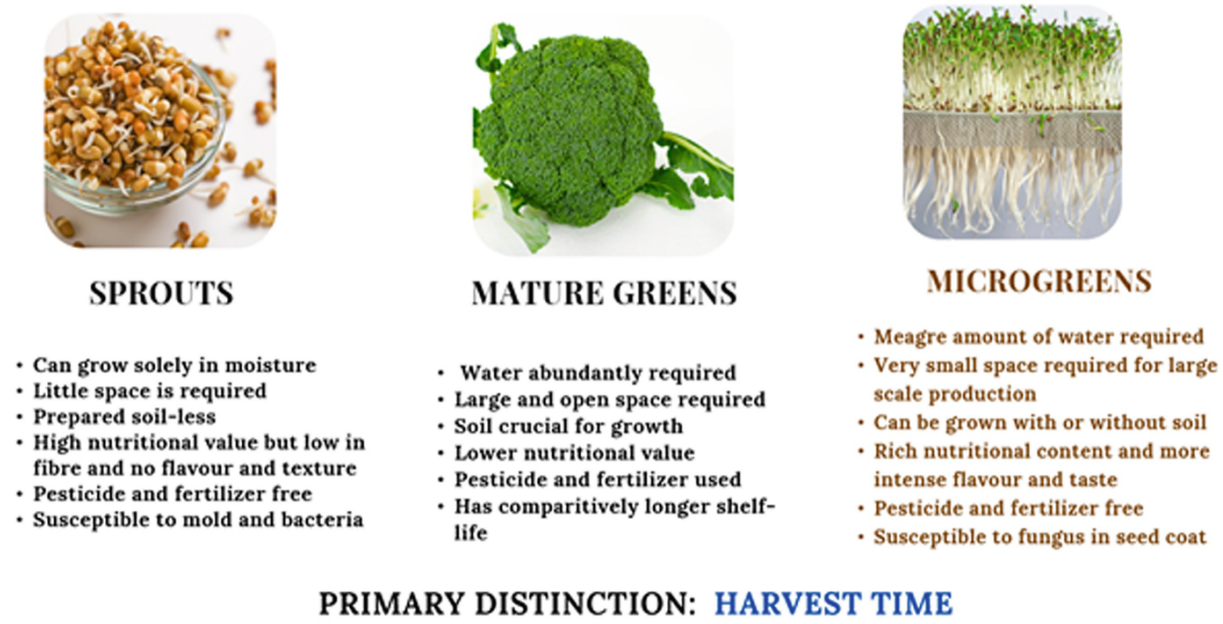


Fig. 1 Characteristic features and differences between sprouts, mature greens and microgreens

Despite their numerous nutritional and health benefits, microgreens face significant challenges during post-harvest storage, which can hinder their commercial viability. This being true for leguminous microgreens as well. Microgreens are particularly prone to rapid deterioration in quality due to factors such as their high surface area, delicate leaves, and rapid respiration rate (Partap et al. 2023). Proper management and storage protocols are crucial to preserve the flavor, texture, and nutritional integrity of microgreens (Commercial Microgreens (MGs): AGRI). Maintaining well-regulated environmental conditions, including optimal light, temperature, and humidity, is essential for their growth and longevity (Sharma et al. 2023). A constant low temperature, ideally around 4 °C, is critical for inhibiting microbial growth and extending shelf life (Commercial Microgreens (MGs): AGRI). It is suggested that during germination, microgreens undergo extensive breakdown of seed-storage compounds alongside the synthesis of structural proteins and other cell components, rendering them highly perishable. Strategies such as proper packaging, storage temperature optimization, controlled environments, and lighting adjustments can all contribute to

preserving the nutrient quality of microgreens. A better understanding of the importance of these factors in retaining the nutritional value of microgreens may also help ensuring consumer satisfaction and promoting food safety (Zhanga et al. 2021). By employing effective post-harvest treatments, producers can enhance the marketability and nutritional benefits of microgreens, thereby contributing to the overall quality and accessibility of fresh produce. Addressing these challenges through proper handling and storage practices is essential for maximizing the shelf life and marketability of microgreens in the commercial sector.

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2 Legumes as Microgreens

Fabaceae, known for its diversity and agricultural significance, encompasses a staggering 20,000 species, making it the third largest among angiosperms and the second largest among domesticated plants (Sehaole 2022). Despite this vast diversity, only a select eleven species are cultivated for various purposes (Maroyi 2023; Usman et al. 2022). Legumes, a prominent subset of the Fabaceae family, are valued for their rich nutritional profile, comprising carbohydrates, proteins, dietary fiber, phytochemicals, essential minerals, and oils. Notably, they contain isoflavones and antioxidants, contributing to their reputation as a beneficial dietary component (Adebo et al. 2022; Gu et al. 2022). With their high protein content and relatively low lipid levels, legumes hold promise in combating poverty and malnutrition, particularly amongst vegetarian populations of the world and in developing regions where protein-energy malnutrition (PEM) is prevalent. Furthermore, legumes serve as excellent nitrogen fixers, owing to their symbiotic relationship with *Rhizobium* bacteria, offering agricultural benefits by reducing the need for synthetic fertilizers and regulating air pollution. Tran and Nguyen (2009) highlighted the dual role of legumes in environmental sustainability and nitrogen regulation, emphasizing their importance in crop cultivation. By delivering economic advantages through reduced fertilizer costs and environmental benefits through nitrogen fixation, legumes emerge as pivotal crops in achieving sustainable agriculture and ensuring food security (Sehaole 2022). When discussing legumes, it is of utmost relevance to know that legume cultivation is often limited by environmental factors such as climate

suitability and soil type, which are critical for optimal yield and growth (Shevchenko et al. 2022). For maximizing yield while minimizing environmental impacts, especially developing cultivation and implementing technologies can also play a significant role (Cernay et al. 2018). Moreover, agricultural practices and policies greatly influence the selection of legume species for cultivation, depending on the availability of cultivars that are economically feasible and have adapted better to local conditions compared to other species (Abobatta et al. 2021). According to research, grain legumes such as lentils and chickpeas are preferred due to their resilience to low nitrogen input conditions, which can lead to increased cereal yield during crop rotation practices (Cernay et al. 2018). The perennial legumes, on the other hand, frequently encounter constraints such as the need for specific machinery that can handle their soil interaction without jeopardizing the plant's root system and growth habits, which are crucial for its nutrient intake and overall health (Shevchenko et al. 2022).

Legume microgreens, thus, have the potential to be a better alternative when compared to fully mature plants. Since they require less space and water than mature plants, they make an ideal choice for urban and small-scale farming and can be grown indoors easily without the use of pesticides, thereby reducing water runoff (Saleh et al. 2022). Nutritionally, legume microgreens are far superior compared to their mature counterparts. They have higher concentrations of vitamins, minerals, phytochemicals, and antioxidants, the compounds that are vital for boosting overall health and preventing chronic diseases. The antioxidant activities of microgreens have been reported to contribute to reducing inflammation, boosting cardiovascular health, and potentially combating cancer (Tallei et al. 2024). Research showed that small-seeded legume microgreens are intriguing new sources of nutritious food that may be used to fortify staple foods with nutrients, minerals, and bioactive chemicals. The various legume microgreens grown are enlisted in Table 1 with their nutritional content, phytochemical composition and substrates used for their cultivation.

Table 1 The various legume microgreens grown with their nutritional content, phytochemical composition and substrates used for their cultivation

Microgreens	Scientific name	Nutritional content	Substrate/ Cultivation systems	Phytochemical Composition	References
Chickpea	*Cicer arietinum*	Carbohydrates, Proteins, Fat, Fiber, Vitamin B_5 (Pantothenic Acid), Vitamin B_6 (Pyridoxine)	Vermiculite, Coconut Coir	Carotenoids, Lutein, Isoflavones	Jukanti et al. (2012), Kariasa et al. (2024)
Fenugreek	*Trigonella foenum-graecum*	Potassium, and Minerals (K, Ca, Cl, K, P, S, Cu, Fe)	Cocopeat	Phenolic, Flavonoids, α-tocopherol	Dhull et al. (2021), Ebert (2022), Ramya et al. (2020)
Mung bean	*Vigna radiata*	Carbohydrates, Protein K, P, Ca, Zn, Fe, Cu, Mn, Mg, Na	Soil Based Cultivation	Phenolics, Flavonoids, Carotenoids, Tocopherols, Ascorbic Acid	Priti et al. (2021), Ramya et al. (2020)
Green pea	*Pisum sativum*	Mg, P, S, K, Ca, Mn, B, Fe, Ni, Cu, Zn, Mo	Soil, Cocopeat, peat, perlite, Cellulose	Chlorophyll, Carotenoids, Phylloquinine, Phenolics, Antioxidants	Citua et al. (2020), Ebert (2022), García-Tenesaca et al. (2024), Poudel et al. (2023, 2024)
Lentil	*Lens culinaris*	Ca, P, K, Zn, Mg, Mn, Na, Fe	Coco Peat: Vermiculite: Sand (2:1:1)	Phenolics, Flavonoids, Carotenoids, Tocopherols, Ascorbic Acid	Priti et al. (2021, 2022)
Cowpea	*Vigna unguiculata*	Carbohydrates, Proteins	Soil, Cocopeat, Water	Ascorbic Acid, Chlorophyll, Phytic Acid	Eswaranpillai et al. (2023), Sharma et al. (2022)
Horse gram	*Macrotyloma uniflorum*	Vitamin C, Crude Protein	Soil, Cocopeat, Water	Chlorophyll, Beta Carotene, Flavonoids, Phenolic Acids	Arya (2021), Eswaranpillai et al. (2023), Rajaprakasam et al. (2023)

The therapeutic advantages of secondary metabolites such as phytoestrogens, triterpene saponins, and condensed tannins have drawn interest from official medicine in addition to veterinary and traditional medicine. Legume-derived sprouts and microgreens may also be included in wheat products to boost health-promoting

effects that can be gained from secondary plant metabolites (Cornara et al. 2016). These substances have been linked to several health benefits, including anti-menopausal, anti-inflammatory, anticancer, anthelmintic, antidiabetic, anti-hypercholesterolemia and cardioprotective properties (Butkutė et al. 2018). Quercetin, a type of flavonoid, is renowned for its potent anti-oxidative, anti-proliferative, anti-diabetic, anti-inflammatory, anti-viral, and anti-carcinogenic properties (Deepika and Maurya 2022). The compound is reported to be present in abundance in pea leaves and microgreens. Lupin leaves had a higher concentration of total flavonoids than microgreens, although lupin microgreens had a higher quantity of genistein (Klopsch et al. 2018).

During germination, there is a tremendous increase in the content of polyamines like putrescine (PUT), spermine (SPM), agmatine (AGM), cadaverine (CAD), and spermidine (SPD) in species such as alfalfa, fenugreek, and lentils. Consuming these polyamines through food is nutritionally beneficial, particularly, SPD and SPM seem to have neuroprotective and cardioprotective properties. AGM may even pass through the blood-brain barrier and, thus, can be taken in large doses without having a negative impact on health, helping with symptoms of illnesses of the central nervous system, such as severe depression. Different tissues of Leguminosae family germinated seeds express diamine oxidases. The enzyme is mostly expressed in the hypocotyl and root systems of soybean sprouts. Bean sprout activity has been observed primarily in the cotyledons and in all sections of fava beans except the cotyledons. In the hypocotyl and root of chickpeas, higher levels of CAD or PUT were found to be linked with increased enzyme activity. Supplemental enzymes that catalyse biogenic amines' oxidative deamination can be taken as dietary supplements (Kralj et al. 2020).

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3 Genetic Enhancement of Leguminous Microgreens

With the burgeoning population, limited land available for agriculture, worldwide climate change, a variety of biotic and abiotic stresses; there is a lot of pressure on conventional methods of crop breeding and domestication. Traditional breeding approaches for the enhancement of legume crops with respect to its

quality, nutrition, and productivity alone may not be able to meet the ever-increasing global demand. Conventional breeding involves crossing two or more plants with relevant characteristics to produce a new generation with the desired combination of traits of interest. These approaches have by far allowed scientists to develop legume cultivars with various improvements like increased yield, resilience to disease, tolerance to drought, and nutritional benefits like increased protein, iron, zinc, and other vital micronutrients (Afzal et al. 2020; Raina et al. 2023).

Molecular breeding, marker-assisted selection, genetic engineering techniques, and genome editing technologies are examples of modern breeding procedures (Raina et al. 2023). The possibility of structural variation and functional genomics has been made possible by the sequencing and resequencing of significant legume species. Reference genome sequences of nearly thirty grain legume species are available (Zhang et al. 2022). Researchers can now access information more quickly and accurately with the help of next-generation sequencing (NGS) and other molecular techniques like genotyping, high density genetic linkage maps, expressed sequence tags (ESTs), single nucleotide polymorphisms (SNPs), quantitative trait loci (QTLs) identification, transcription factors integrated into breeding technologies, and marker development. Genome sequencing, RNA sequencing (transcriptome sequencing), and DNA sequencing (re-sequencing) are useful tools for improving and developing legume strategies (Afzal et al. 2020). The efficiency and resolution of genetic mapping and marker-trait association studies are being improved by the availability of large-scale genomic resources and low-cost, high-throughput genotyping technologies. Above all, the application of molecular breeding techniques has produced enhanced lines for several legume crops, including peanuts and chickpea (Pandey et al. 2016).

Genetic biofortification focuses on developing genotypes capable of accumulating microelements through overexpression techniques (Singh 2023). Modern genomic techniques can speed up a variety of processes, including trait mapping, molecular breeding, gene identification, marker generation, and productivity gains in microgreens (Fig. 2). The integration of low-frequency variations, the sequencing information method, and precise phenotypic variation would be beneficial in selecting the most suitable accessions with desirable key features such as nutritional

attributes, biotic and abiotic stress tolerance, and biomass components (Gupta et al. 2023).

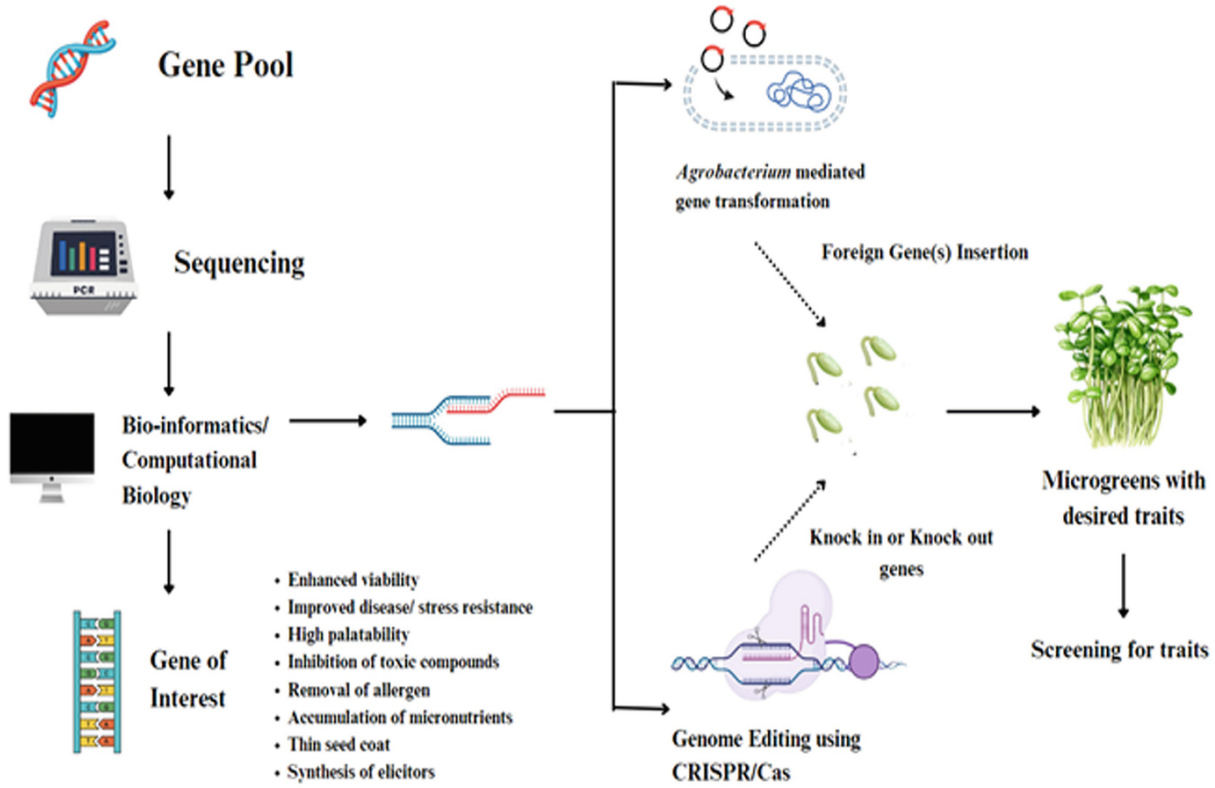


Fig. 2 A flow chart illustrating the biotechnological process of enhancing vital traits in microgreen crops, involving gene identification, and storage using omics and bioinformatics approaches followed by the transfer of candidate gene into the plant using transformation or genome editing

3.1 Genetic Engineering as a Potential Solution and the Traits Targeted

Genetic engineering offers transformative potential for enhancing leguminous crops by targeting specific genetic traits for improving nutritional content, disease resistance, and stress tolerance. For example, by employing strategies that use ‘omic’ technologies, enhancement of nutrient profiles such as increased protein, mineral, and vitamin content has been achieved for legumes. The same strategy can be well utilized for microgreens as well, which differ from their mature counterparts only in their harvest time when taking cultivation into account (Popoola et al. 2019). Targeting resistance to pathogens and potential control points for microgreen production, genetic engineering can help enhance shelf life of microgreens (Riggio et al. 2019). Genetic modification aims

to enhance the crops' resilience against environmental stresses such as salinity and drought, along with their capacity for nitrogen fixation, which is essential for maintaining sustainable soil conditions (Zhukov et al. 2013).

Leguminous microgreens can be genetically engineered to have superior nutritional content, disease resistance, stress tolerance, increased growth and yield, and phytoremediation abilities. Biofortification stands out as a fundamental strategy for augmenting the nutritional quality of plants. It is recognized as a cost-effective and feasible approach, holding promises for addressing nutritional deficiencies in populations with limited access to diverse diets or commercially fortified foods (Andersson 2017). All the legumes play pivotal roles in ensuring food and nutritional security, especially for the vegetarian populations of the world and fostering environmental sustainability. However, major cereal crops lead in the integration of genomic technologies into breeding programs (Varshney et al. 2019). Despite the importance of grain legumes in the food chain, efforts to enhance their nutritional content have been limited (Roorkiwal et al. 2021). Agronomic crop management, traditional breeding, and genetic engineering both via genetic transformation and genome editing approaches offer avenues for biofortification or improving the nutritional quality of legume crops as well. Agronomic bio fortification particularly stands out for its applicability to a wide range of crops and cultivars, making it a versatile strategy for addressing nutritional challenges (Poudel et al. 2023, 2024).

For instance, perennial legume microgreens and sprouted seeds have higher zinc contents, which makes them possible sources of zinc fortification for food. Additionally, when compared to intact pea seeds, sprouting increased the availability of Zn and Mg in pea seeds and Fe and Ca in soybeans and *Faba* beans (Luo et al. 2014; Urbano et al. 2006). It is therefore possible to speculate that the microgreens and sprouted seeds of perennial legumes are a rich source of Zn and Fe, and that their bioaccessibility may even be enhanced (Butkutė et al. 2018). Butkutė et al. (2017) and Wu et al. (2012) found that *Trifolium* species, chickpeas, and other pulses have significantly increased concentrations of primary isoflavones during germination. Similarly, a study on the biofortification of selenium and salicylic acid in lentils not only reported a slight increase in ascorbic acid, flavonoids, anthocyanins, and selenium,

but a significant increase in proline content as well (Hawrylak-Nowak et al. 2018). Another example is the biofortification of *Vigna unguiculata* (cowpea) with potassium iodide to increase iodine content, resulting in a significant enrichment of iodine levels, particularly in the plant (Giordano et al. 2023).

The potential gene(s) responsible for plant architecture, trait regulation, flexibility, stress resistance, adaptability, and post-harvest modifications, along with their molecular mechanisms, may be identified for further investigations and research (Partap et al. 2023). These traits can be genetically engineered. Shelf life and plant storage features can be enhanced using genome editing as a possible venture. For example, through transgenic and molecular approaches, the microgreens of chickpeas and pigeon peas have been explored for improvement in their shelf life and plant storage (Sharma et al. 2023). Association mapping (AM) has been utilized in common beans to identify QTLs or gene(s) associated with protein, iron, and zinc levels. This information can be manipulated to achieve overexpression of the genes to enhance the protein, iron and zinc levels respectively in common bean microgreens as well (Salgotra and Stewart 2022). Figure 2 shows a flow chart to represent the biotechnological way for enhancing the vital traits in microgreen crops. The first part includes finding the gene of interest from the genome pool and using omics and bioinformatics approaches to store it for further considerations. The next part incorporates the transferring of the desired genes to the targeted plant using genome editing or transformation approaches.

3.2 Other Approaches for Biofortification of Leguminous Microgreens

The biofortification of sprouts and microgreens is a fascinating area of study as it enhances the nutritional content of sprouts by increasing a health-promoting micronutrient in the sprout tissue and eliciting an effect on secondary metabolites. Enhancing growth metrics and nutritional content in microgreens can be achieved through various approaches. Efforts have been made to enhance the nutritional quality of crops through altering the basic physical conditions required for photosynthesis, viz light, temperature, CO_2, water, and humidity (Lobiuc et al. 2017; Gupta et al. 2015). The same can be very well applied to our leguminous superfood. For instance, manipulating light wavelengths with LED lighting can

boost the accumulation of health-promoting compounds like anthocyanins, phenolic compounds, and vitamins in microgreens, offering a non-invasive method to enhance their nutritional value (Lobiuc et al. 2017). Altering light spectra using LEDs significantly influences nutrient content and growth in microgreens, presenting an effective strategy for enhancing their nutritional value and yield-repetition (Samuolienė et al. 2019). Altering the sound frequency (using ultrasound) boosts the overall yields of microgreens, as well as the rates at which seeds germinate and produce roots and hypocotyl. It has been reported that ultrasound can help microgreens acquire beneficial compounds like GABA and phenolic compounds as well as accelerate the breakdown of lipids, proteins, and carbohydrates (Bhabani et al. 2024). *Trigonella* microgreens were developed under various photoperiods and temperatures, revealing increased plant height and total chlorophyll content under T1 conditions. The predominant phenolic profiles were gallic acid, p-coumaric acid, and apigenin. The sugar profile showed increased concentrations of myo-inositol, glucose, fructose, xylose, maltose, and sucrose. The microgreens were abundant in amino acids and proline concentration surged under T1 growth conditions (Kumar et al. 2024).

Furthermore, an enhanced antioxidant capacity may result from the activation of phenolic compound synthesis in plants via a variety of elicitation processes. When lentil sprouts were treated with 15 mM H_2O_2, their phenolic content increased by nearly 65% (over control). Furthermore, applying temperature stress for one hour at 4 and 40 °C improved the phenolic content and antioxidant potential of lentil sprouts. In addition to being used as elicitors, microwaves, gamma, and UV radiation can also be used to lessen seed contamination and associated health issues (Alclade et al. 2022). This is a comparatively simpler method to enhance the phytocompounds present in the plant species and can very well be applied for microgreen enhancement.

The application of protein hydrolysates and biostimulants has shown promise in improving fresh yield, biomass increase, soluble protein levels, and total free amino acid content (El-Nakhel et al. 2021). Furthermore, seed inoculation with microbial consortia has demonstrated significant enhancements in grain yield and growth parameters due to synergistic bioinoculant effects (Gupta et al. 2015). Leveraging plant growth-promoting rhizobacteria (PGPR)

and rhizobial inoculation can optimize nodulation, nutrient uptake, and overall plant vigor in microgreens, leading to improved growth performance (Swarnalakshmi et al. 2020). Additionally, dual inoculation with compatible microbial consortia has been found to enhance symbiotic efficiency and grain yield, particularly under stress conditions (Kumawat et al. 2021). One of the best methods available for enhancing the biotechnological synthesis of plant secondary metabolites (PSMs) is elicitation. The process of elicitation involves the addition of trace amounts of elicitors, which induces or enhances the manufacture of secondary metabolites in plants. Elicitors can be categorized as biotic (such as polysaccharides originating from plant cell walls, microorganisms, and glycoproteins) or abiotic (such as metal ions, inorganic chemicals). Salts added during sprouting are one of the more readily available and affordable methods of inducing plant defense among abiotic elicitors (Mahto et al. 2022).

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4 Challenges and Future Perspective

Riggio et al. (2019) emphasized that food safety is of paramount importance when considering microgreens, including leguminous types due to favorable condition being created for microbial pathogens. The irregulated microbial growth may lead to foodborne illnesses. Infact microgreens have faced several recalls due to pathogenic infection in US and Canada. *Salmonella* being the cause of three recalls, while *Listeria* being the cause of four of them (Turner et al. 2020). Using various bacterium-biological control strategies, some writers have claimed successfully controlling pathogenic bacteria from developing plants (Pratap et al. 2023). For instance, microgreens are disinfected with chlorinated water at concentrations that do not compromise the product's flavor or aroma to stop the growth of microorganisms. (Eliseeva and Simina 2021).

It must be noted that there are no established quality control procedures or standard operating procedures in microgreen farming. Industry-wide guidelines must be set for the selection of seeds, growing material, lighting, humidity, temperature, and nutrition management. Ensuring consistency in flavor, texture, and nutritional content can be achieved by regular quality control (Zhang et al. 2021). Microgreens are more susceptible to physical

harm than adult green leaves because they are immature and delicate since they are extremely tender. When subjected to stress, freshly cut microgreens deteriorate quickly (Abaajeh et al. 2023). Also, a milder, less astringent, sour, and bitter flavor in microgreens holds the key to customer acceptance of the plant group (Singh 2023). Thus, the quality post-harvest and shelf life are still compromised when commercial production of microgreens are considered,

A lot can be considered when we discuss the scope of improvement in leguminous microgreens for future perspective. Endophytic bacteria such as *Bacillus megaterium* (strain RmBm31) have demonstrated the potential to enhance growth and stress tolerance in host plants, suggesting their role in bolstering resilience in microgreens. The genome of the bacterium has been fully sequenced and is identified to possess a wide range of genomic features linked to plant growth promotion, positive modification of root architecture, increase in the biomass of the plant, and production of volatile organic compounds (Dahmani et al. 2020). These genes can thus be engineered into the plant to enhance the mentioned traits.

For legumes, such as common bean seed, all three components, namely, the seed coat, cotyledons, and embryo, can be the target for improvement as opposed to cereals. A thorough research attempt has been made for the biofortification of Fe, Zn, and P in the common bean and to study the interaction of the minerals Fe-Zn and Fe and P in the seed coat. A hypothesis suggesting that a significant gene on B04 is associated with Fe accumulation in the seed coat was proposed which might open potential avenues for gene cloning and characterization (Blair et al. 2013). Further studies revealed the overlapping regions of quantitative trait loci (QTL) for Zn accumulation (Blair et al. 2013). When talking about the microgreen stage, all of this can be applied even to them for biofortifying Zn, Fe and P. In soybean, a legume utilized as vegetable oil and preferred for its high-quality protein, the level of provitamin-A, oleic acid, and other protein contents of seed can be increased with the expression of the bacterial *PSY* gene (beta-carotene) (Schmidt et al. 2015). In fact, based on CRISPR-Cas12a nuclease system in soybean, a 50% editing rate and 30% transformation efficient greenhouse ready edited plant has been described (Liang et al. 2023).

Literature suggests that no significant work has been done on leguminous microgreens towards targeting some specific traits for their genetic advancement. All the challenges they face during their commercialization are yet to be combatted, providing tremendous scope in research and development. Latest techniques, such as RNA interference (RNAi), transgenic breeding, and genome editing include the new methodologies pivotal for crop biofortification strategies. For diversity analysis and identification of potential genotypes for desirable traits, a number of DNA-based molecular markers are available, which includes AFLP (Amplified fragment length polymorphism), SSR (Simple sequence repeats), SNP (Single Nucleotide Polymorphisms), and microRNA-based SSR (Gupta et al. 2023).

In particular, the exceptional qualities of hydrogels based on biopolymers are emphasized for potential use as sprout and microgreen growth substrates in the future. Hydrogel-based substrates derived from functional biopolymers combined with nutrients and antimicrobials will be able to accomplish two goals at once: biofortifying trace elements during seed germination and growth and preventing microorganism infestation in sprouts and microgreens. The fact that hydrogels are recyclable and structurally robust makes them potentially a practical means of achieving sustainability (Du et al. 2022).

5 Conclusions

Microgreens, an innovative approach holds promise in aiding human in achieving UN SDGs by 2030s, providing us with an easily cultivable, sustainable, and nutritious food supplement. However, like any other innovation, it has been facing challenges during its production and commercialization in a range of species due to its short shelf-life and post-harvest quality deterioration. Genetic enhancement has the potential to tailor them for the development of microgreens that are more resistant to pests, diseases, and adverse environmental conditions, in addition to their high nutrition levels, thereby increasing crop yields and ensuring food security. This ability to withstand stress could also reduce the reliance on pesticides and other chemical inputs, promoting sustainable farming practices. Additionally, genetic enhancement to improve the taste and texture of microgreens will make them more appealing to consumers and encourage greater consumption.

However, microbial susceptibility of microgreens has posed a major hurdle in its commercialization and has resulted in several recalls. They are even reported to cause allergic reactions in some people due to their organoleptic properties, but much evidence is not there yet. In a nutshell, the field is still open to a lot of exploration and research. By harnessing the power of genetic innovation, one can not only bolster food security but also cultivate healthier eating habits, ultimately contributing to a more sustainable and resilient food system for generations to come. Thus, the emergence of leguminous microgreens offers a viable and sustainable solution to address worldwide concerns surrounding agriculture, food security, and health.

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Genetic Engineering: A Potential Tool in Enhancing the Quality and Shelf Life of Leguminous Microgreens

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Abbreviations

ROS Reactive Oxygen Species

GMO Genetically modified organism

CRISPR Clustered Regularly Interspaced Short Palindromic Repeats

NCD Non-communicable disease

LOX Lipooxygenase

VOC Volatile organic compounds

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1 Introduction

Preservation of shelf life and fortification of nutrition are two essential aspects while dealing with storage of a food produce. Chemical preservation of a food product poses challenges with respect to the concentration of the chemical used and whether any adverse effects are observed while consuming the food. Microgreens are delicate in nature and loaded with several essential nutrients. Legume microgreens, more specifically, are rich in proteins and minerals. As these young plants are constantly growing, it is essential to preserve their nutrient composition before they mature into a plant. Cold temperatures and chemicals may be able to arrest their nutrition partially and therefore a more prudent means of preservation is necessary. Genetic engineering may have the answers to these questions. GMOs as they are popularly called, have been in the forefront of food technology where chemical methods of preservation have performed poorly. This chapter aims to discuss the merits of genetic engineering in enhancing the nutritive value in legume microgreens.

Legumes have an important place in nutrition in many parts of the world owing to their high protein, carbohydrate, dietary fiber and high-energy source food content (Bubelova et al. 2018). In addition, legumes are rich in secondary metabolites, such as antioxidants, flavonoids, carotenoids and chlorophyll. These compounds,

which cannot be synthesized in the body, have the potential to counteract nutritional deficiencies (Klopsch et al. 2018).

2 Nutrition and Shelf Life of Microgreens Grown Traditionally

Generally, seeds and germinated products have different levels of crude fat content in seeds and sprouts and moderate levels of ash and carbohydrates. However, microgreens have higher levels of ash and carbohydrates.

There is a lack of comprehensive studies on the chemical makeup of seeds, sprouts, or microgreens of perennial legumes. The findings from Butkute et al. (2018) regarding fat levels in alfalfa seeds in sprouts are consistent with those reported by Márton et al. for the same respective counterparts. Perennial legume seeds generally have a higher protein content compared to most grain legume seeds, with the exception of soybean, yellow and white lupin. Perennial legumes showed higher or similar levels of crude fat and ash compared to grain legumes typically used in food, except for soybeans and peanuts (Hedley 2001).

The total polyamines accumulated because of the germination of lentil, fenugreek, alfalfa, and daikon radish. Polyamine content in all three legume sprouts showed a significant increase. These are regarded as nutritionally advantageous. The formation of microgreens resulted in decreased levels of nutritionally beneficial polyamines. In terms of the changes in legume content, this behavior was turned around (Kralj et al. 2020).

Selling microgreens when they are still rooted in the growing media maximizes their “shelf life.” To preserve quality, harvested microgreens need to be kept cool. The quality may be retained for more than 14 days, depending on the cultivar and storage circumstances. Microgreens are

exempt from the food code, yet early research indicates that they should be kept at 5 °C in storage.

Following harvest, microgreens can be cleaned to get rid of soil particles and provide a clean product for packaging. Washing greens before packing lowers the initial bacterial load, but it also produces a humid environment that encourages microbial development, so it’s important to remove excess water to stop it. Because washing and dewatering require extra handling that can harm the fragile greens and increase their susceptibility to microbial development, many producers opt not to wash their produce. It can be difficult to remove too much moisture after washing without damaging the fabric. Therefore, maintaining the ideal temperature, moisture content, and environment for microgreens’quality preservation and shelf life while inhibiting the growth of human pathogens and spoiling bacteria requires careful balancing.

The inclusion of microgreens in one’s diet provides several evidence-based health benefits due to their nutritional content, as the existing literature makes clear. Microgreens’ growth, yield, and nutritional composition can depend on factors such as the cultivation method, the intensity and quality of illumination, and the precise makeup of the plant nutrient solution. When implemented responsibly and informed by scientific research, these controlled-condition approaches to microgreen farming stand to benefit public health by increasing access to a nutritious food source (Gupta et al. 2023; Tallei et al. 2024).

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3 Omics Approach—Including Target Genes and Methods

Chronic diseases especially non-communicable diseases (NCDs) are the cause for 74% of all deaths world-wide. Among them cardiovascular disease accounts for 17.9 million deaths which stands first, followed by cancers, respiratory diseases and diabetes (World Health Organisation, WHO). Vitamins treat most of the ailments and diseases and minerals with other medications and patients are advised to take nutritious food. Abundant data recommends that consumption of (3–4 cups) help to mitigate the possibility of chronic disorders. Recent studies suggest that microgreens of vegetables and legumes contain various health-promoting elements and can be used as an efficient replacement for mature vegetables (Butkute et al. 2018; Rizvi et al. 2023).

Phytochemicals, nutrient content, shelf life and antioxidant capacity of plants are dependent on the genetic makeup and ecological conditions. Mishra et al. (2021) group has analysed the levels of phytochemicals, protein, micro and macronutrients including the antioxidant capacity of 20 different genotypes each of lentil and mung bean microgreens. The study identified that phytochemical concentration particularly flavonoids and carotenoids of L830 genotype of lentils and MH810 genotype of mungbean were superior. Hence, from the available diverse genotypes it is imperative to identify the most favourable genotype comprising the desirable traits like high nutrition, shelf life, yield and taste. Most advanced methods have evolved that apply in the recent discoveries of food research. They include foodomics approaches that comprises genomics, transcriptomics, metabolomics, proteomics and nutrigenomics. These tools help in screening and selection of the desirable genotype by unravelling the intricacy of the foodome and the biological system (Priti et al. 2021).

Limited studies have focused on the food-omics strategies for microgreens research and yet to be employed on legume microgreens. Recently from three various

samples of microgreens, three strains (GTAL407, GTA L409, and GTAL411) of Listeria monocytogenes was isolated and a complete genome sequence (MinION and MiSeq) and explored for the microbial resistance genes (Naushad et al. 2022). Similarly whole genome sequence data and metagenomic sequencing data of bacterium species isolated from microgreens was characterized to study the micro-biome and resistance related genes (Moon et al. 2022). Few metabolomic analyses of few other microgreens like broccoli and Brassica microgreens (Alrifai et al; 2021; Partap et al. 2023). However, 'Omics' approaches are yet to be employed on legume microgreens to characterize and unravel the differentiation of origin, nutrition content and shelf life from matured plants.

An ideal genotype with multiple desirable traits is important for commercial purposes and sustainable production. However, availability of an individual genotype possessing multiple favourable traits is difficult. Hence, by employing omics data, scientists can edit the genome of microgreens for the enhancement of commercial significant traits. Significant efforts are being made using genetic engineering tools like agrobacterium mediated transformation and genome editing to enhance the genetic makeup related to yield, nutrition and stress tolerance on various legumes (Choudhury and Rajam 2021). Gene editing procedures have been significantly standardised for cowpea, soybean, chickpea including *Lotus japonicus* and *Medicago truncatula* (Bhowmik et al. 2021). However, adopting gene-edited microgreens is challenging because though gene-editing strategies can result in abundant benefits of legume breeding, regulatory obstacles are the serious concern. Considering the increase in population and chronic diseases, public acceptance of GMOs is the important factor for further development.

4 Gene Editing

Clustered Regularly Interspaced Short Palindromic Repeats also called CRISPR is a new method used for different manipulations in gene sequences like creating gene knockouts, inducing specific modifications within a gene, performing multiplex engineering in genomes, or upregulating and downregulating certain genes (Arora and Narula 2017) (Fig. 1).

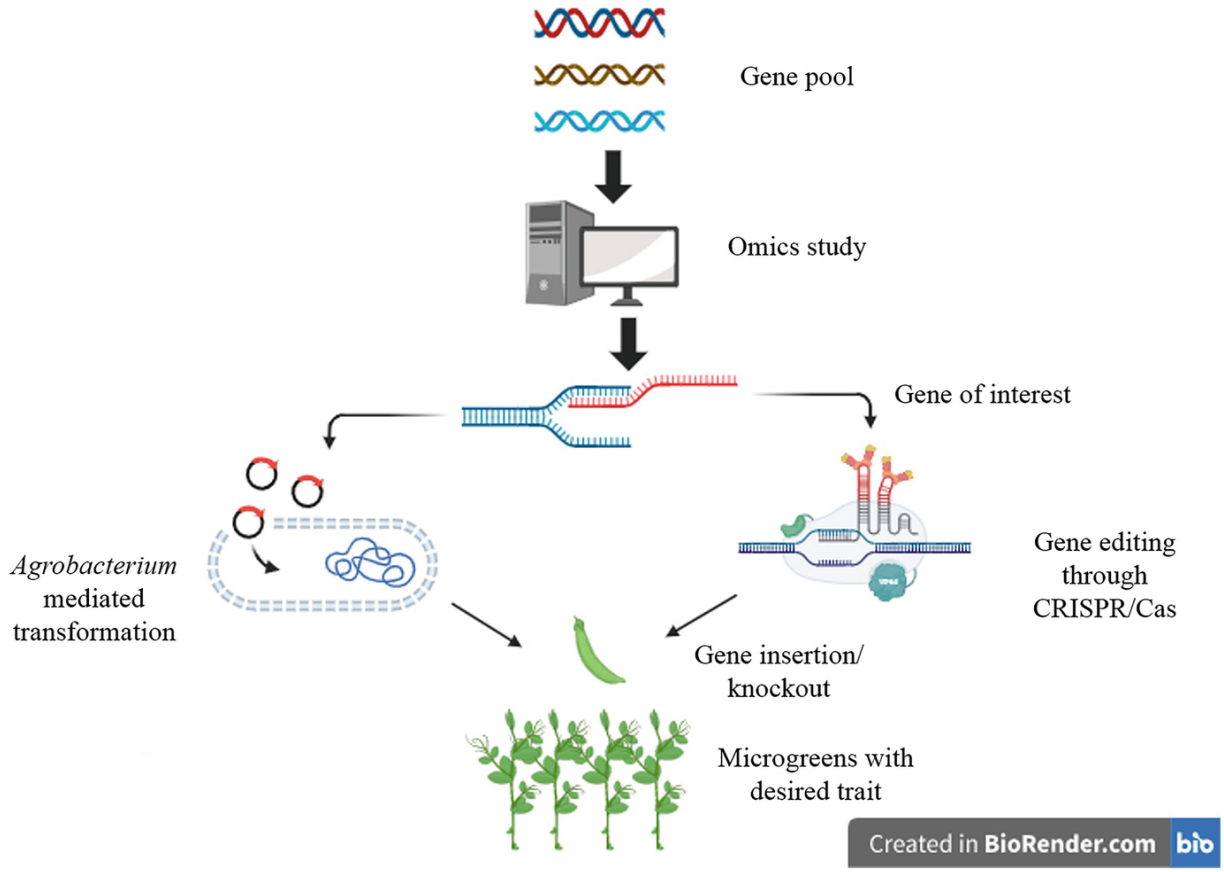


Fig. 1 Schematic representation of gene editing tools in OMICS approach for improvement in legume microgreens

5 What Is CRISPR/Cas9 Technique?

In this technique, two characters occur: a protein called Cas9 (DNA endonuclease, which is RNA-dependent) along

with guide RNA (gRNA). Cas9 binds with gRNA to form a complex. The gRNA contains 20 nucleotides, which are complementary in nature, to the target sequence of DNA (which has to be edited). This gRNA helps in recruiting Cas9. CRISPR/Cas9 uses RNA–DNA interactions to find out the DNA sequence target. In CRISPR/Cas9, for interactions involving DNA and RNA about 18–20 base pair sequence is required. Here, gRNA and Cas9 are bound to a sequence seen at target sequence 3′ end which is known as protospacer adjacent motif, which is a . For example, the sequence of bases 5′-NGG-3′ acts like protospacer adjacent motif for Cas9 in *Streptococcus pyogenes.* This is used for genome editing. Cas9 creates breaks in both strands of DNA in the DNA target and then starts DNA repair by means of non-homologous end joining for producing gene knockouts. Gene insertions and gene modifications are created using homology-directed repair (Huang and Puchta 2019). When non-homologous end joining deletes a DNA strand or inserts a strand of DNA randomly inside a coding region in DNA, frameshift mutation occurs and this induces a gene knockout. For NHEJ, a homologous repair template is not necessary. Hence non-homologous end joining repair mechanism is most popularly used for repairing damage in DNA in all leguminous plants. The homology-directed repair is used to insert known donor DNA sequences in a template into a recipient DNA site at the exact location required to be edited (Jhu et al. 2023).

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6 Use of CRISPR/ Cas9 in Legumes

Gene editing by means of CRISPR/Cas9 has been done in some leguminous plants like *Lotus japonicus, Medicago truncatula*, soybean, chickpea and cowpea .

This technique is also used for inducing mutation in nodulation-associated genes in target specific manner in

these legumes. CRISPR/Cas9 technique was used to examine the nodulation genes' function in *Medicago truncatula* (Curtin et al. 2017). This technique was also used for studies using mutations in five nodule-specific Polycystin-1, Lipoxygenase, Alpha Toxin domain proteins and genes in nitrate peptide family (Trujillo et al. 2019; Wang et al. 2020). Mutations in genes like leghemoglobin, symbiosis receptor-like kinase and Lotus histidine kinase 1-interacting protein were brought about by CRISPR/Cas9 method to develop mutant plants (Cai et al. 2018; Borrelli et al. 2018; Wang et al. 2016, 2019). Functional identification was done by targeted mutation for other genes like SUPERMAN, (Rodas et al. 2021), CYP716A51-1 (bringing about oxidation of triterpenes at C28 position in *L. japonicus*) (Suzuki et al. 2019).

Soybean (*Glycine max*) (used for for oil and protein production) was one of the initial plants used for improvement of genes by the CRISPR/Cas9 method (Bao et al. 2020). Its genome consists of many duplicated genes and thus cannot be researched by conventional techniques.

Bao et al. (2019) caused mutation of 4 soybean genes *GmSPL9* in by making use of CRISPR/Cas9 method. This resulted in differing amounts of nodes expressed on the main stem and different branches. These nodes are responsible for the growth of the plants and making the soil fertile. The method of CRISPR/Cas9 could also alter traits in seeds like seed oil component changes (Do et al. 2019), bad beany flavor in seed products of soybean (Wang et al. 2020), increase quantity of isoflavone and bring about immunoresistance to soybean mosaic virus (Zhang et al. 2020). Mutation of genes responsible for producing seed storage protein which is helpful in breeding consumable soybeans were generated by Li et al. (2019) using this technique.

Che et al. (2021) created a construct of CRISPR/Cas9 which was expressed under the transcriptional regulation of soybean elongation factor promoter which showed greater editing activity. Using this scientist used the *VuSPO11-1* meiosis gene to create plants, which undergo only asexual mode of reproduction to develop hybrids plants.

Lentils (*L. culinaris* Medik.), the crops classified as pulse crops are consumed for proteins, carbohydrates, dietary fibres, minerals, vitamins, and antioxidant, anti-cancer, anti-atherosclerotic compounds of phenolic origin (proanthocyanidins, some flavonols and flavan-3-ols) (Tallei et al. 2024). Lentils occur among the top six legumes that are harvested in the form of dry seeds. Environmental adaptation of lentils and their stability to different climates with enhanced meal quality has been done by CRISPR/Cas9 technique by introducing novel alleles other than those existing in the conventional germplasm pool. *Pisum sativum (*pea) seeds are rich in protein especially lysine content. Pea seeds are made up of protein (20–25%) and starch (50%) (Gatel and Grosjean 1990). Inspite of precious nutritional content of pea seeds, they are not widely used in food because of metallic and astringent taste brought about by a combination of methoxypyrazines, alcohols, ketones, aldehydes volatile organic compounds and saponins of non-volatile nature like triterpene glycosides. Genes responsible for these traits are being edited by CRISPR/Cas9 to make good quality pea foods. Volatile organic compounds (VOCs) are synthesized enzymatically during cultivation of plants, harvest and transportation of these plants, and when pea plant products are stored. This occurs by means of lipoxygenases (LOX). These LOX enzymes convert linolenic acid and linoleic acid polyunsaturated fatty acids to form fatty acid hydroperoxides (Porta and Rocha-Sosa 2002). These hydroperoxides are broken by hydroperoxide lyases

and aldehyde dehydrogenases, to produce different VOCs. CRISPR/Cas9 technique is now being used to mutate *LOX* in a targeted fashion to reduce the undesirable taste of pea products. Mutants of soybean that have their LOX2 genes deleted by CRISPR/Cas9 technology have showed improved taste compared to LOX1/3 deletion mutants (Davies et al. 1987). These mutant plants where LOX2 gene was deleted, the yield of seed was found to be normal. Further, there was also an increased disease resistance in these mutant plants (Pfeiffer et al. 1992) (Table 1).

Table 1 Studies depicting application of CRISPR-Cas9 in legume plants

Plants	**Genes targeted by CRISPR-Cas9 technique**	**Effects caused**
Soybean (*Glycine max*)	*GmSPL9*	Increase in number of nodes expressed on the main stem and different branches, thus increasing growth of the plants and making the soil fertile
Soybean (*Glycine max*)	*VuSPO11-1* meiosis gene	Undergo only asexual mode of reproduction to develop hybrids plants
Pea (*Pisum sativum*)	mutate *LOX*	to reduce the undesirable taste of pea products by helping mutated LOX to modify Volatile organic compounds (VOCs)
Soybean (*Glycine max*)	mutate *LOX*	Enhanced flavour, increased diseases resistance
Rice plant	OsSWEET13 gene	Resistance to bacterial blight disease
Tomato	SlMlo1	Resistance to powdery mildew
Rice plant	OsERF922 and OsSEC3A genes	Fungal blight disease

7 Conclusion

To summarise, although legumes microgreens are well documented for their rich nutritional value and high

protein content, the importance of genetic engineering in enhancing the value needs to be explored. This report attempts to describe the works on genetic engineering on microgreens on other plants and extends the same to validate the effectiveness of genetic engineering of legume microgreens. Much is left to be explored in this regard.

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Genetic Engineering and Genome Editing for Enhancing Nutritional Quality of Legumes

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Abbreviations

Zn Zinc
Fe Iron
B Boron
Cu Copper
Ni Nickel
Mo Molybdenum
ZRT Zn regulated transporters
QTLs Quantitative Trait Loci
RFO Raffinose family oligosaccharide
% Percent

1 Introduction

Malnutrition has become a significant concern worldwide, leading to various health issues stemming from inadequate consumption of essential micronutrients such as vitamins and dietary supplements (Khoury et al. 2014). Approximately per year 20 million newborns are with lower than average baby weight due to issues caused by micronutrient deficiency, resulting in neonatal mortality (Kumar and Pandey 2020). The significant consumption of calorie-dense staples without sufficient micronutrient intake result in a condition known as hidden hunger, particularly prevalent among populations residing in developing countries (Dawson et al. 2019). Among different nutrient deficiency iron deficiency, zinc deficiency, folate deficiency etc. is very common and pulses are recognized for their significant protein levels, providing cost-effective substitutes for animal-based protein sources. Food legumes, including chickpeas (*Cicer arietinum* L.), common beans (*Phaseolus vulgaris* L.), lentils (*Lens culinaris*), mung beans (*Vigna radiata* L.), peas (*Pisum sativum*), and pigeon peas (*Cajanus cajan* L. Millsp.), are abundant sources of essential amino acids and dietary micronutrients. Besides, underrated legumes like cowpea (*Vigna unguiculata*) and Bambara ground nut (*Vigna subterranean*) can significantly enhance the nutritional value of our diets. Consequently, they hold promise in addressing the escalating issues associated with malnutrition.

Legumes (microgreens), classified under the Fabaceae family of flowering plants, encompass around 800 genera and approximately 20,000 species (Stagnari et al. 2017). Leguminous crops are essential for sustainable agriculture as they contribute to soil health via the release of nutrient-rich organic materials and symbiotic nitrogen fixation. The mechanism of acquiring, moving, and accumulating minerals in the seeds of legumes is believed to be regulated by a complex set of genes, although many of these genes remain unidentified. Several studies have identified genes responsible for the absorbation and mobilization of iron (Fe) and zinc (Zn) from various parts of the plant to the seeds. For instance, research by Palmer and Guerinot (2009), Xiong et al. (2012), and Sen Gupta et al. (2017) has shed light on genes involved in Fe uptake and mobilization, while studies by Palmgren et al. (2008) and Urwat et al. (2021) have focused on genes associated with Zn transport. However, there are likely additional genes yet to be discovered that play crucial roles in these processes. Draft sequence of genome and transcriptome assemblies have been produced in over 35 legumes, as documented by Bauchet et al. in 2019. These have broadened the research prospects of future

legume research. Along with this available information, enhancing yield potential along with biofortification presents a multifaceted challenge that could be addressed through the adoption of innovative techniques such as genetic engineering and precision breeding methods like gene editing. Besides enhancing the concentration of micronutrients, this method can furthermore be utilized to target the elimination of anti-nutrients or promoters of intake, thereby increasing the bioavailability of minerals (Carvalho and Vasconcelos 2013; Garg et al. 2018).

Genetic transformation through *Agrobacterium* and particle are both widely used gene engineering techniques. However, *Agrobacterium*-mediated transformation has become more popular since its inception, particularly in plant transformation due to its affordability and ease of access, and its capability of facilitating single- or low-copy transgene insertions. Although the first transgenic pulse crops was reported and published by Köhler et al. (1987) in *Vigna aconitifolia*, progress in legume transformation has been relatively slow. Up to 2021, the number of approved genetic alteration events occurred in legumes is 49, with 42 of these events belonging to soybean (*Glycine max*) (https://www.isaaa.org). However, this is less explored in legumes. Again gene editing involves the utilization of engineered nucleases and cellular DNA repair mechanisms to achieve accurate and specific modifications to the genome of an organism. Despite being discovered three decades ago, the application of meganucleases for generating specific double-stranded breaks during gene editing has been limited. This is primarily due to the low target site frequency present in most genes (Rouet et al. 1994). Efforts to enhance the efficiency of gene editing systems saw significant advancement with the introduction of programmable zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) (Bibikova et al. 2002; Li et al. 2011; Zhang et al. 2011). However, the true discovery in this groundbreaking technology occurred with the successful adaptation of RNA-guided Cas9 nuclease, derived from prokaryotic (type II) clustered regularly interspaced short palindromic repeats (CRISPR) adaptive immune system, for eukaryotic gene editing (Cong et al. 2013; Mali et al. 2013). This book chapter is intended to focus on a comprehensive update that grows with the chronological progress occurred in genetic engineering and gene editing in legumes with special emphasis on nutritional quality improvement.

2 Nutritional Status and Health Benefits of Legumes

Legumes with their nutritional detail have been provided in Table 1.

Table 1 Nutritional quality in different legumes

Crop name	Scientific name	Seed Protein Content (~)	Deficient Amino Acids	References
Chickpea	*Cicer arietinum*	17–28%	Methionine, cysteine, threonine and valine	Hulse et al. (1991), Badshah et al. (2003), Manan et al. (1984)
Lupin	*Lupinus albus* L.	35–44%	Alanine and tryptophan	Lucas et al. (2015), Duranti et al. (1979), 2008)
Lentil	*Lens culinaris*	20–31%	Methionine and cysteine	Urbano et al. (2007), Khazaei et al. (2019)
Pigeon pea	*Cajanus cajan* Millsp.	20–22%	Methionine, cysteine and valine	Bressani et al. (1986), Saxena et al. (2002)
Cowpea	*Vigna unguiculata*	15–25%	Methionine	Horax et al. (2004), Gerrano et al. (2019), Martos-Fuentes et al. (2015)
Mung bean	*Vigna radiata*	21–32%	Methionine and cysteine	Anwar et al. (2007)
Faba bean	*Vicia faba*	26–41%	Methionine	Picard (1997), Crépon et al.. (2010)
Pea	*Pisum sativum*	14–31%	Methionine, cysteine and tryptophan	Mossé et al. (1987), Tzitzikas et al. (2006), Özer et al. (2012)
Soybean	*Glycine max* L. Merr.	Up to 40%	Methionine, cysteine, threonine and lysine	Hou et al. (2009), Sharma et al. (2014), Patil et al. (2017)

The nutritional profiles of underutilized legumes like Bambara groundnut (*Vigna subterranean*), Jack bean (*Canavalia ensiformis*), Lima bean (*Phaseolus lunatus*), and sword bean (*Phaseolus edulis*) were examined in a study conducted by Soetan and Adeola (2018). Plants such as *Cassia hirsuta* L. offer a rich source of essential nutrients, including protein, lipids, potassium, fiber, carbohydrates, and energy, which can effectively meet the nutritional requirements of humans. Velvet bean (*Mucuna pruriens*) is recognized for its exceptional nutritional composition, boasting optimal levels of total protein, lipids, fiber, carbohydrates, energy, potassium, zinc, calcium, manganese, phosphorus, and magnesium (Kala and Mohan 2010). When compared to soybeans, certain species of Bauhinia and *Canavalia gladiata* have greater protein content. Moreover, both Bauhinia and Delonix are notably abundant in different types of amino acids. Additionally, tree bean seeds (*Parkia timoriana*) and winged bean seeds (*Psophocarpus tetragonolobus*) demonstrate a wide array of fatty acids. Transfer of gene for biofortification from such genotypes especially from

underutilized legumes into elite genotypes lacking them will be very much convenient at current scenario. Grain legumes, although rich in minerals, contain various anti-nutritional elements such as phytic acids (phytate or PA), oxalates, and tannins, which bind to minerals, reducing their bioavailability and leading to unhealthy issues. Several studies have identified the existence of phytic acids (Shi et al. 2018; Pramitha et al. 2021), RFOs (Roorkiwal et al. 2021), oxalates (Shi et al. 2018), and tannins (Petroski and Minich 2020) in pulses, all of which exhibit anti-nutritional properties.

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3 Molecular Mechanisms Behind Mineral Transport in Legumes

Numerous investigations have sought to delineate gene families responsible for mineral transport from various plant tissues to seeds. While significant progress has been made in understanding genes and proteins facilitating primary zinc (Zn) and iron (Fe) uptake from soil, we aim to elucidate the transport mechanisms of mainly Zn and Fe specifically in legumes.

The absorption and transportation of zinc (Zn) primarily occur through the uptake of Zn^{2+} across root cell plasma membranes. Transporters like ZIP (Zinc Iron Permease) play a crucial role in facilitating Zn absorption and transport from roots to seed (Palmgren et al. 2008). Key protein families involved in Zn transport are ZIP, Zn regulated transporters (ZRT), IRT-like proteins, MTP (Metal Tolerance Protein) and HMA (Heavy Metal ATPases) (Gupta et al. 2016). Various ZIP genes have been found across different plant species, including *Arabidopsis*, Soybean, *M. truncatula*, and *Phaseolus vulgaris* (Assunçao et al. 2010; Moreau et al. 2002; Lopez-Millan et al. 2004; Astudillo et al. 2013). Symplastic and apoplastic routes both affect the Zn fluxes in the shoot. Zn is taken up by apoplastic transport, which is less selective than symplastic transport and occurs via contact between the cell wall and the plasma membrane. Selectivity and distribution of nutrients are regulated by symplastic transport (Gupta et al. 2016). Zinc is sequestered into vacuoles by transporters such as MTP1 and ZIF1, which belong to the cation diffusion facilitator family (CDF); zinc is then mobilized from vacuoles by NRAMPs (Haydon and Cobbett 2007). Zn intake into xylem is facilitated by HMA transporters, where it is transported as Zn^{2+} or in complexes within the xylem (Palmgren et al. 2008). While ZIP family members govern Zn^{2+} entry into leaf tissue and the phloem, YSL carries zinc into the phloem and delivers it as the Zn-NA complex to seeds (Waters and Grusak 2008). Recent studies in

common bean have highlighted the importance of genes like IRT1, FRO1, and Ferritin 1 in Fe and Zn uptake, transport, and signaling under Fe/Zn stress. Additionally, HA2, NRAMP1, ZIP2, and GLP1 genes in *Phaseolus vulgaris* have shown high responsiveness to Zn uptake (Urwat et al. 2021).

Iron (Fe) absorption and distribution to legume roots are critically dependent on proteins such IRT1 (iron-regulated transporter), FRO2 (ferric reductase oxidase 2), and HA2 (H^+-ATPase) (Roorkiwal et al. 2021). Furthermore, the absorption of Fe(III)-phytosiderophores by the transporter protein YS1, which is located in the plasma membrane, is crucial for the acquisition of Fe (Curie et al. 2001). Numerous genes encoding Fe transporter protein families have been identified by expression analysis of multiple legume species, including *Arachis hypogaea*, *Medicago truncatula*, *Glycine max*, *Lens culinaris*, and *Cicer arietinum* (Xiong et al. 2012; Tejada-Jiménez et al. 2015; Lopez-Millan et al. 2004; Qin et al. 2017; Sen Gupta et al. 2017; Parveen et al. 2016). The specific Fe sensing mechanisms are yet unknown, despite the finding of different networks at transcriptional level controlling iron uptake and distribution (Thomine and Vert 2013). High-affinity transporters like ZIP and YSL are involved in metal unloading from the xylem, whereas IRT1 mainly helps with Fe absorption (Roorkiwal et al. 2021). Oligopeptide transporter (OPT3) might be essential for precise long-distance Fe importation into phloem partner cells and for correct Fe transfer from shoots to roots (Kumar et al. 2017b). FRD3 mutants, for example, show aberrant Fe localization within the plant due to abnormalities in Fe homeostatic control (Green and Rogers 2004). Studies on pea mutants such as bronze (brz) and degenerative leaves (dgl) emphasize the significance of Fe control in the development of shoots and seeds (Cary et al. 1994; Grusak et al. 1996). Plants contain nicotianamine (NA), a non-protein amino acid that is essential for the translocation of metals, including Mn, Fe, Cu, and Zn (Pich et al. 1994; Pich and Scholz 1996). YSL genes have a role in transporting of metal complexes to a long-distance with NA, seed formation, and metal remobilization from senescent leaves (Di Donato et al. 2004).Several other genes for other micronutrients like P, Cu, K, Mg, seed proteins, vitamins have been reported. In legumes like soybean (Kumar and Pandey 2020), chickpea (Vandemark et al. 2018; Karaca et al. 2020), common bean (Ray et al. 2014; Murube et al. 2021), cowpea (Gerrano et al. 2022; Sodedji et al. 2022), lentil (Vandemark et al. 2018), mung bean (Kumar and Pandey 2020), pea (Dissanayaka 2019; Klein et al. 2020) a significant amount of genetic variations has been observed for micronutrient content and mapping of QTLs for such transporter genes has also been achieved till date. Understanding about these transporter

genes of minerals will facilitate further legume breeding towards biofortified variety development.

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4 Biofortification Strategies

4.1 Conventional Approach

Taking underutilized legumes under consideration for biofortification of legumes would be a great option for breeders. While attempts have been made using traditional hybridization and other breeding techniques to achieve certain goals, the anticipated outcomes have not been realized. Limited success has been observed in the crossbreeding of underutilized legumes, with only a handful of documented successful instances. On *Cajanus cajan*, African yam bean there is no report on successful breeding line development yet (Hickey et al. 2017; Bekkering and Tian 2019). Numerous underutilized legumes face reproductive challenges such as embryo abortion, which hinder genetic progress. To overcome these constraints, techniques like micro-propagation and tissue culture can be employed to generate living haploid plants (Cullis and Kunert 2017).

4.2 QTL Mapping

In the past decade, there has been a notable shift in the approach to generating genomic resources in diverse legumes, enabling the elucidation of genetic mechanisms governing crucial traits, such as grain micronutrient and protein content. The advent of easy to use novel single-nucleotide polymorphism (SNP) markers has facilitated the application of Genome-Wide Association Studies (GWAS) in marking genomic regions or haplotype assemblies rich in micronutrient across various grain legumes (Diapari et al. 2014; Khazaei et al. 2017; Wu et al. 2020; Diaz et al. 2020). Several QTLs for Fe, Zn, P, S, K, Se, Mn content have been reported in different legumes like common bean, pea, lentil, mung bean, soybean, chick pea etc. (Bohra et al. 2015; Singh et al. 2017; Sab et al. 2020; Wu et al. 2020; Ma et al. 2017; Park et al. 2019). Again several studies have uncovered QTLs responsible for seed protein content of chick pea, pea, pigeon pea, soybean, common bean, ground nut etc. (Jadhav et al. 2015; Campa et al. 2011; Sarvamangala et al. 2011; Obala et al. 2020; Sebolt et al. 2002; Whiting et al. 2020).

4.3 MicroRNA Exploitation

In addition to protein-coding genes, microRNAs (miRNAs) are appealing prospects for improving the micronutrient content of grains (Sunkar and Zhu 2004). Through their involvement in a variety of plant biological and metabolic processes, notably development, the synthesis

of cell walls, and stress responses, miRNAs provide a promising means of enhancing grain micronutrients in food crops through the regulation of gene expression (Chen 2009). Numerous miRNAs have been found to control differentially expressed genes that control the absorption, mobilization, and stability of nutrients, guaranteeing an adequate supply without causing toxicity. These genes mostly impact micronutrients such as Mn, B, Mo, Cu, Zn, and Ni (Patel et al. 2017). Furthermore, it has been noted that miRNAs are important regulators of nutrient homeostasis and responses to plant stress (Paul et al. 2015). However, there is little evidence regarding their role in legume micronutrient homeostasis, with the exception of *Medicago truncatula* and *Phaseolus vulgaris* (Valdes-Lopez et al. 2010; Naya et al. 2014). The expression of genes involved in nutritional homeostasis is influenced by miRNAs, which are notable for their reactivity to nutrient deficits like Cu and Fe (Abdel-Ghany and Pilon 2008; Sunkar et al. 2006; Naya et al. 2014; Pant et al. 2008; Waters et al. 2012). Moreover, miRNAs regulate the expression of genes associated to stress in response to stressors such as exposure to heavy metals and Mn toxicity (Valdes-Lopez et al. 2010; Zhou et al. 2012; Li et al. 2013; Zeng et al. 2014). Notably, reactions to sulfate, P, and Cu deficit in *Arabidopsis* and *Brassica* demonstrate how nutrient scarcity induces miRNA expression (Sunkar et al. 2006, 2007; Naya et al. 2014). These discoveries on the regulation of nutrient homeostasis by miRNA may provide new approaches to improving efforts at legume biofortification (Blair et al. 2013).

4.4 Agronomic Approach

Agronomic interventions enhance the efficiency of mineral fertilizer application and promote the solubilization and mobilization of mineral elements in soil (White and Broadley 2009). Fertilizer application can elevate soil micronutrient levels, addressing deficiencies in humans (Cakmak 2008). Implementing agronomic practices such as intercropping can enhance the efficiency of phosphorus (P), iron (Fe), and zinc (Zn) uptake, resulting in heightened yield and enhanced grain quality (Xue et al. 2015). Moreover, soil attributes like elevated pH levels, presence of calcite, organic matter, and excessive concentrations of other ions impede the availability of micronutrients for agricultural plants (Alloway 2009).

4.5 OMICS

Genomics, transcriptomics, proteomics, metabolomics studies also offer great opportunities for nutritional quality improvement in legumes (Fig. 1).

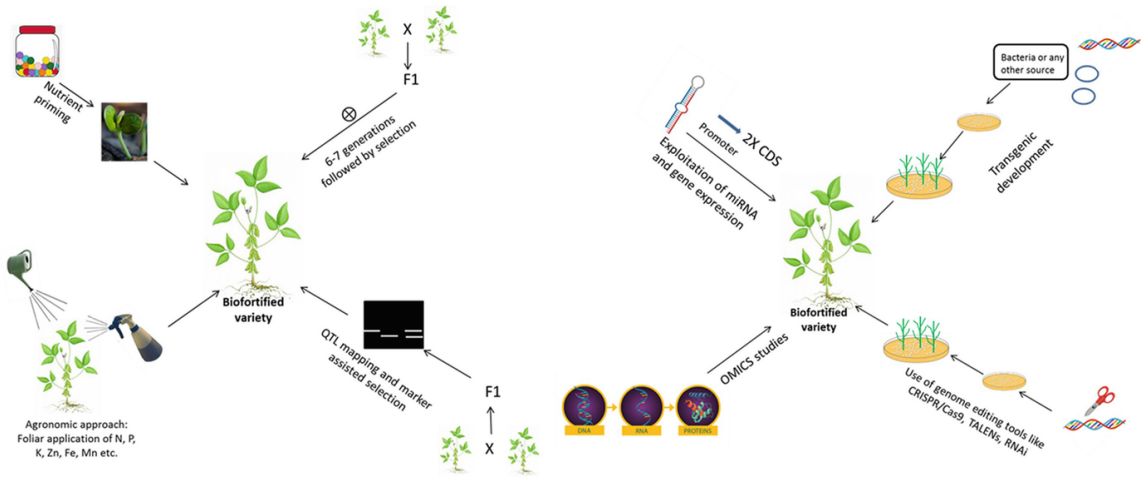


Fig. 1 Strategies behind biofortified variety development

Next Generation Sequencing (NGS) offers the capability to sequence genes associated with nutrients in different leguminous crops (Rychel-Bielska et al. 2020). By harnessing data from whole-genome sequencing (WGS), one can pinpoint genes linked to nutrient accumulation. These genes can then be integrated into breeding programs to enhance crop traits (Hawkins and Yu 2018). Earlier, model legumes like *Medicago truncatula* and *Lotus japonicus* have furnished genomic insights, facilitating progress in breeding initiatives and technologies such as omics and genome editing. These advancements enable the extraction and transfer of desirable traits to enhance the nutritional composition of legumes (Bohra et al. 2020). Genomic sequence availability of different crops like soybean, pigeon pea, chick pea, lentils, lupine, and mungbean etc. also helps in breeding for biofortification (Desta and Ortiz 2014; Annicchiarico et al. 2017).

Until recently, the primary methods for gene expression studies included northern hybridization, serial analysis of gene expression (SAGE), microarrays, among others. However, significant progress has occurred in recent years thanks to improvements in tools of analytical studies and NGS technologies (Molina et al. 2008). RNA sequencing (RNA-seq), accomplished through cDNA sequencing, has emerged as a viable alternative for gene expression studies (Carpentier et al. 2008). This method has become one of the most efficient tools extensively employed to analyze nutrient-responsive genes and those linked with adaptation.

Advancements of proteomics and metabolomics with the aids of proteome analysis, protein profile, metabolite pool detection studies have enough potential to facilitate further nutritional quality improvement of legumes (Coen et al. 2008; Fahy et al. 2007; Jackson et al. 2010; Gulzar and Minnaar 2017).

4.6 Gene Editing and Genetic Modification Approach

The detailed description of these two approaches has been given in the following section.

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5 Genetic Engineering in Legumes for Nutritional Quality Improvement

Plant transformation, a critical component of genetic engineering methodologies, encompasses a series of steps, commencing with the identification of a desired gene, followed by its delivery, integration into plant cells, expression, culminating in the development of a mature plant. Genetic engineering, alongside traditional and molecular breeding methods, has expanded possibilities in legume research aimed at biofortification. *Agrobacterium* and particle bombardment mediated gene transfer have been schematically represented (Fig. 2).Temporal benchmarks of legume genetic engineering has been shown (Fig. 3). Factors like genotypes with different morphogenic genes; explants chosen, culture media, *Agrobacterium* strain etc. are the critical factors affecting successful plant transformation. For example;

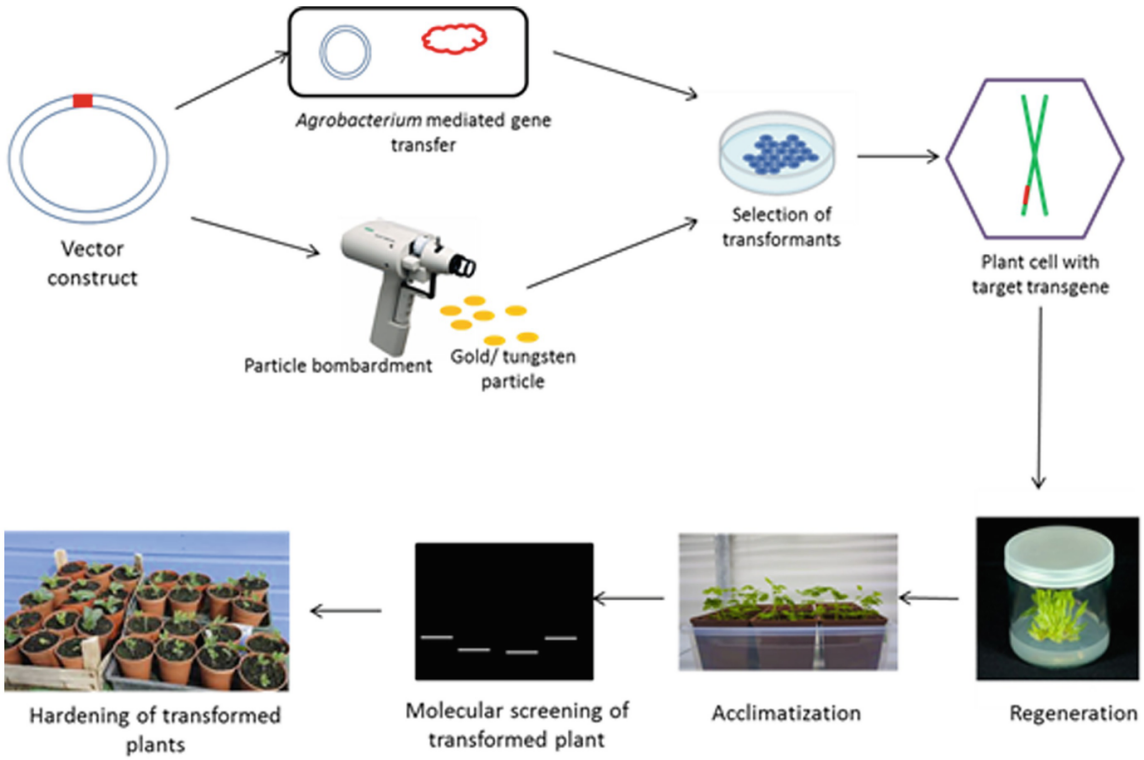


Fig. 2 Transgenic development through *Agrobacterium* and particle bombardment mediated gene transfer

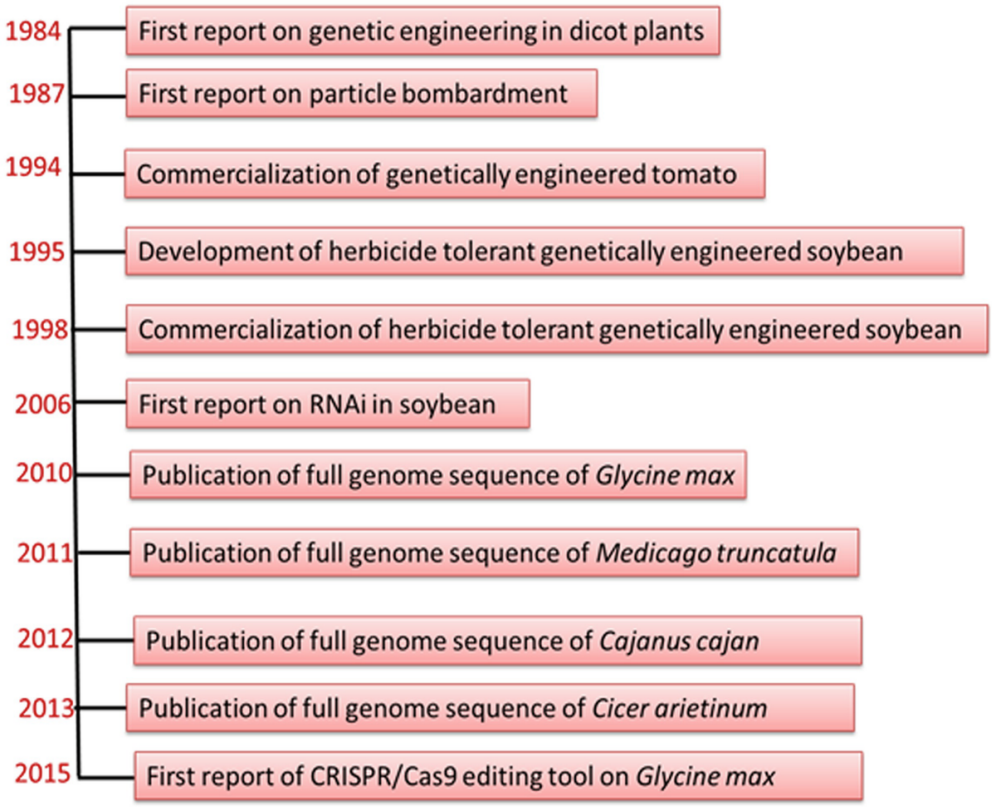


Fig. 3 Temporal benchmarks on genetic engineering on legumes

5.1 Genotype

The genotype of the donor plant significantly impacts legume transformation success, with many reports limited to specific genotypes. This constraint represents a significant bottleneck in legume transformation. While initial reports mainly identified certain genotypes like Peking in soybean as highly susceptible to Agrobacterium infection, subsequent research has expanded the repertoire of successful genotypes, including Williams 82, Bert, Jack, and Maverick (Hinchee et al. 1988; Yamada et al. 2012; Pareddy et al. 2020). ICCC37, ICCV 89,314 varieties of chick pea, ICPL 87, ICPL 87,119 of pigeon pea, Pusa Komal of cowpea have demonstrated to facilitate achieving elevated transformation frequencies (Ganguly et al. 2020; Karmakar et al. 2019; Kumar et al. 2017a, b). However, this reliance on genotype can be mitigated by exploring alternative methods like in floral dip, planta technique, or seedling dip transformation, which do not rely on tissue culture.

5.2 Morphogenic Genes

Genes such as Leafy Cotyledon1 and 2 (Lec1, Lec2), Wuschel (WUS), Baby Boom (BBM), Agamous-Like15 (AGL15), and Wuschel-Related

Homeobox (WOX) have been documented to enhance plant regeneration processes (Lowe et al. 2016; Kausch et al. 2019). The significance of these genes has been further confirmed across numerous dicot species and crops, underscoring their broad relevance in plant regeneration (Nagle et al. 2018; Gordon-Kamm et al. 2019; Kong et al. 2020). Nevertheless, the direct application of these genes in legume genetic transformation has yet to be realized.

5.3 Explants

The cotyledonary node, extracted from tiny seedlings, has been extensively utilized in various experiments and deemed a perfect explant for transforming several legume species (Das 2018; Sarkar et al. 2019). Conversely, half-seed explants have emerged as efficient cotyledonary explants, significantly (at the rate of 35%) boosting transformation rate in soybean (Karthik et al. 2020). Exploration of embryonic axes, plumular meristems, and entire trifoliates or leaflets has demonstrated the potential to further enhance transformation efficiency to a significant degree (Paes de Melo et al. 2020; Ganguly et al. 2020; Jiang et al. 2019).

5.4 Culture Media

The component of the culture medium represents other rate-limiting criteria in in vitro regeneration, prompting ongoing extensive research aimed at optimizing its formulation. The addition of thiol compounds like DTT, $_{L}$- cysteine, and sodium thiosulphate to the co-cultivation medium reduces the activities of wound- and pathogen-responsive enzymes such as peroxidases and polyphenol oxidases in plants. This inhibition also limits browning of tissue and enhances the delivery of T-DNA (Liu et al. 2008; Yadav et al. 2017).

5.5 Agrobacterium Strain

The choice of Agrobacterium strain can significantly impact the transformation frequency (Tichá et al. 2020). Various *Agrobacterium* strains have been evaluated for legume transformation, including EHA105, EHA101, LBA4404, GV3101, AGL1, and C58, which are commonly employed in research protocols.

Exploring transformation systems other than tissue culture and manipulating morphogenic regulators offer potential solutions to challenge the obstacles in transgenic biology. Implementing a joined approach involving both *Agrobacterium*-mediated transformation and particle bombardment holds promise for enhancing transformation frequency. Techniques such as pollen bombardment, pollen electroporation, pollen magnetofection, pollen-tube transformation, and

nanotechnology-based approaches warrant application across various genera. Table 2 describes the achievements of legume transformation for nutritional quality improvement.

Table 2 The genetic engineering work in legumes for nutritional quality improvement

Crop	Explants	Genotype/cultivars	Gene delivery system	Target character/gene	References
Soybean	Cotyledonary node	Jack	*Agrobacterium*-mediated	Omega-3 fatty acid desaturase	Flores et al. (2008)
	Hypocotyl	Heinong44	*Agrobacterium*-mediated	Increased oleic acid	Wang and Xu (2008)
	Cotyledonary node	HN66	*Agrobacterium*-mediated	Improved phosphorous efficiency	Zhou et al. (2014)
	Immature cotyledon	Pusa 16	*Agrobacterium*-mediated	γ-tocopherol methyltransferase expression	Arun et al. (2014)
	Cotyledonary node	Maverick	*Agrobacterium*-mediated	Silencing of raffinose synthase 2	Valentine et al. (2017)
	Half-seeds	Kwangan	*Agrobacterium*-mediated	beta-carotene	Kim et al. (2012)
		Jack	Particle bombardment mediated	beta-carotene, oleate and protein content vy bacterial phytoene synthase overexpression	Schmidt et al. (2015)
	Half-seeds	Kwangan	*Agrobacterium*-mediated	Cysteine	Kim et al. (2012)
	Immature zygotic cotyledon	Jack	*Agrobacterium*-mediated	Methionine and cysteine by overexpressing maize zein	Dinkins et al. (2001)
	Cotyledonary node	Misuzudaizu and Bert	*Agrobacterium*-mediated	Methionine and cysteine by *Arabidopsis* cysta-thionine γ-synthase expression	Hanafy et al. (2013)
			Agrobacterium-mediated	Stearidonic acid (STA) by borage Delta 6 desaturase and the Arabidopsis Delta 15 desaturase co expression	Eckert et al. (2006)

Crop	Explants	Genotype/cultivars	Gene delivery system	Target character/gene	References
		Jack	Particle gun bombardment	Flavonoids	Yu et al. (2003)
			Agrobacterium-mediated	Vit-E	Van Eenennaam et al. (2003)
				Arachidonic acid	Patent-US7943816 B2
Chickpea	Half-embryonic axis	PBA HatTrick	*Agrobacterium*-mediated	Fe biofortification	Tan et al. (2018)
	Half-embryonic axis	HatTrick	*Agrobacterium*-mediated	Fe biofortification by OsNAS2 and CaNAS2 expression	Das Bhowmik et al. (2019)
	Single cotyledon and half embryo	Pusa 362 (IC296139)	*Agrobacterium*-mediated	Zn, P, Cu, K, Fe, Mg by CaCKX6 expression in roots	Khandal et al. (2020)
	Half-embryonic axis	HatTrick	*Agrobacterium*-mediated	Nicotianamine synthase (NAS) and ferritin (FER) for Fe fortification	Tan (2018)
Pigeon pea	Cotyledonary node	ICPL 87	*Agrobacterium*- and particle bombardment mediated	Overexpression of dihydrodipicolinate synthase to improve lysine content in seeds	Thu et al. (2007)
Common bean	Apical meristematic region of embryos	Olathe	Particle gun bombardment	Brazil nut's methionine-rich storage albumin gene improves methionine and cysteine levels	Aragao et al. (1999)
Lupin	Sliced embryonic axis with cotyledons	Warrah	*Agrobacterium*-mediated	Increased methionine content due to overexpression of sunflower seed albumin gene	Molvig et al. (1997)

6 Genome Editing in Legumes for Nutritional Quality Improvement

Artificial nucleases like as zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR/CRISPR-associated protein) can be used to alter genomes. Cas9 is an effective technique for changing the expression of a gene without changing the genotype's genetic composition (Bortesi and Fischer 2014; Jaganathan et al. 2018). Utilizing contemporary genome editing methodologies will facilitate the creation of non-genetically modified (non-GMO) plant varieties possessing specific traits. This advancement holds the potential to enhance yield capacities in challenging environments and elevate nutritional standards. A recent investigation showcased the combined application of CRISPR/Cas9 and TALENs technologies, resulting in the creation of a series of effective mutant-plant lines within *Glycine max* and *Medicago truncatula* (Curtin et al. 2018). A brief description of these tools has been given below;

6.1 CRISPR/Cas9

Modern improvement in molecular genome based editing, such as the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR/Cas9) technology, have revolutionized genetic modification by allowing precise modifications to almost any genomic sequence with a known Protospacer Adjacent Motif (PAM), thus enabling the creation of desired phenotypes. This technology has successfully tackled major challenges in crop improvement, including enhancing resistance to plant diseases and insect pests, increasing tolerance to climate changes, and improving both yield and quality across nearly 20 different plant species.

Initially identified as a microbial adaptive immune system in bacterial cells like *Escherichia coli*, the CRISPR/Cas9 system operates through a 20 base pair-long single guide RNA (sgRNA), designed to complement the target gene sequence, allowing for precise binding. Cas9, acting as a nuclease, induces single or double-stranded breaks, triggering the cell's DNA repair mechanisms, such as Non-Homologous End Joining (NHEJ) or Homologous Directed Repair (HDR), often facilitated by the presence of a donor DNA sequence (Ishino et al. 2018; Cui et al. 2018). The stringent PAM requirement of the CRISPR/Cas9 technology poses a major obstacle since it limits the flexibility of target site selection. Researchers have recently used various orthologs of Cas9 with greater PAM compatibility and longer guide RNA (gRNA) to overcome this restriction and enhance the scope of targeting. (Makarova et al. 2015; Manghwar et al. 2019; Puchta 2017; Van Eck 2020).

Additionally, because gRNAs have very small seed lengths, worries about off-target binding and cleavage by Cas9 have been voiced. Cas9 proteins possess two nuclease domains, HNH and RuvC, responsible for cleaving the complementary and non-complementary strands of the target DNA, respectively (Jinek et al. 2012). Nickase variants of Cas9 have been created by adding point mutations in the active centres of either the RuvC (Cas9 D10A nickase) or HNH (Cas9 H840A nickase) domain in order to reduce off-target activity. (Cong et al. 2013; Jinek et al. 2012). The newest tool in the arsenal of genome-editing techniques is prime editing, sometimes referred to as “search and replace” base editing. This method makes use of a template for the intended change, a nickase (H840A Cas9) combined with reverse transcriptase, and a prime editing guide RNA (pegRNA). After the nickase creates a new DNA strand from the template, the reverse transcriptase inserts it into the genome’s nicked location (Anzalone et al. 2019). With prime editing, a wide range of editing operations are possible, such as point mutations, insertions, and deletions, all without causing double-stranded DNA breaks or necessitating donor DNA templates. (Anzalone et al. 2019; Lin et al. 2020) (Fig. 4).

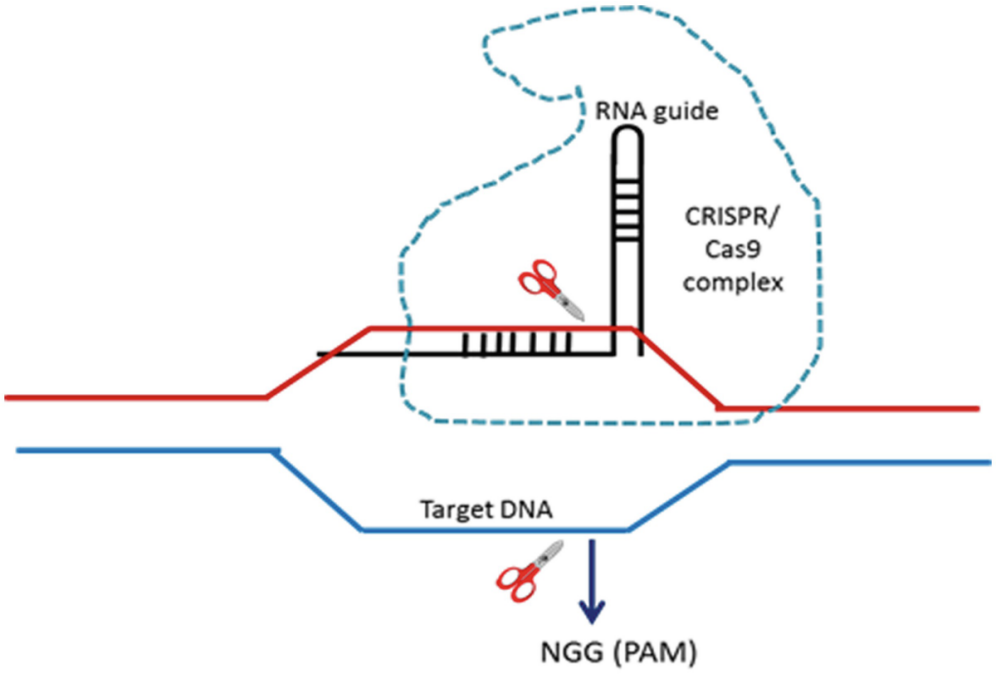


Fig. 4 Schematic illustration of CRISPR/Cas9 constructs and its function

6.2 TALENs

TALEN (Transcription Activator-like Effector nucleases) stands out as a promising gene editing technology, offering a versatile approach to target DNA sequences across a wide array of organisms. Initially

identified in *Xanthomonas* spp., a plant pathogenic bacterium, the TALE (Transcription Activator-like Effector) protein was found to manipulate host plant gene transcription to enhance virulence (Boch et al. 2009). To introduce TALE into a host, a typical type III secretion system is necessary, facilitating bacterial colonization. TALE possesses the ability to bind to virtually any DNA sequence and, when paired with a nuclease, is capable of cleaving double-stranded DNA. Subsequent to this disruption, DNA repair mechanisms such as non-homologous end-joining (NHEJ) or homology-directed repair (HDR) come into play, resulting in precisely induced gene mutations by changing the DNA sequence. TALENs have found extensive utility in enhancing plant genomes for various purposes.

The commonly utilized TALEN system consists of two TALEN units, with each unit comprising DNA binding TALE repeat domains. These domains are made up of one FokI nuclease catalytic domain, two N and C terminal domains, and arrays of highly conserved 30–33 amino acid repeats. Two hyper-variable amino acid residues at positions 12 and 13 control the specificity of each TALE repeat's ability to bind to the A/G/T/C DNA sequence. (Table 3; Streubel et al. 2012). These variable positions are referred to as Repeat Variable Diresidue (RVD). The conserved sequence of the 30–33 amino acid repeat is LTPDQVVAIAS**GGKQALETVQRLLPVLCQDHG, where ** denotes the RVD. The length of the target site corresponds to the number of repeats within the array. Notably, a thymine (T) is situated at the 5′ end preceding the initial base of the TALE repeat.

Table 3 RDV of each TALE repeat domain with their DNA base specificity

NS	NN	NK	IG	NG	HD	NI	RDV
A/C/G/T	G/A	G	T	T	C	A	DNA base specificity

At the end, these domains dimerize to create the functional double-stranded DNA breaks (DSBs), completing the active domain of the final TALEN system. Each FokI nuclease catalytic domain is coupled to one TALE repeat domain. In order to properly promote nuclease-induced double-stranded DNA cleavage, each TALEN unit requires precise binding to the neighbouring target DNA sequence, with a spacer area typically ranging from 12 to 21 bases. These double-stranded DNA breaks (DSBs) set off other DNA repair mechanisms, which have the potential to generate DNA alterations like insertions or deletions (INDELs). It is possible to make exact base substitutions or insertions in the presence of a double-stranded DNA donor template. INDELs result from the positioning of two nuclease-based DSBs on the same chromosome. Once more, if they are on separate chromosomes, they

have the ability to cause chromosomal translocations, which makes genome editing easier (Fig. 5).

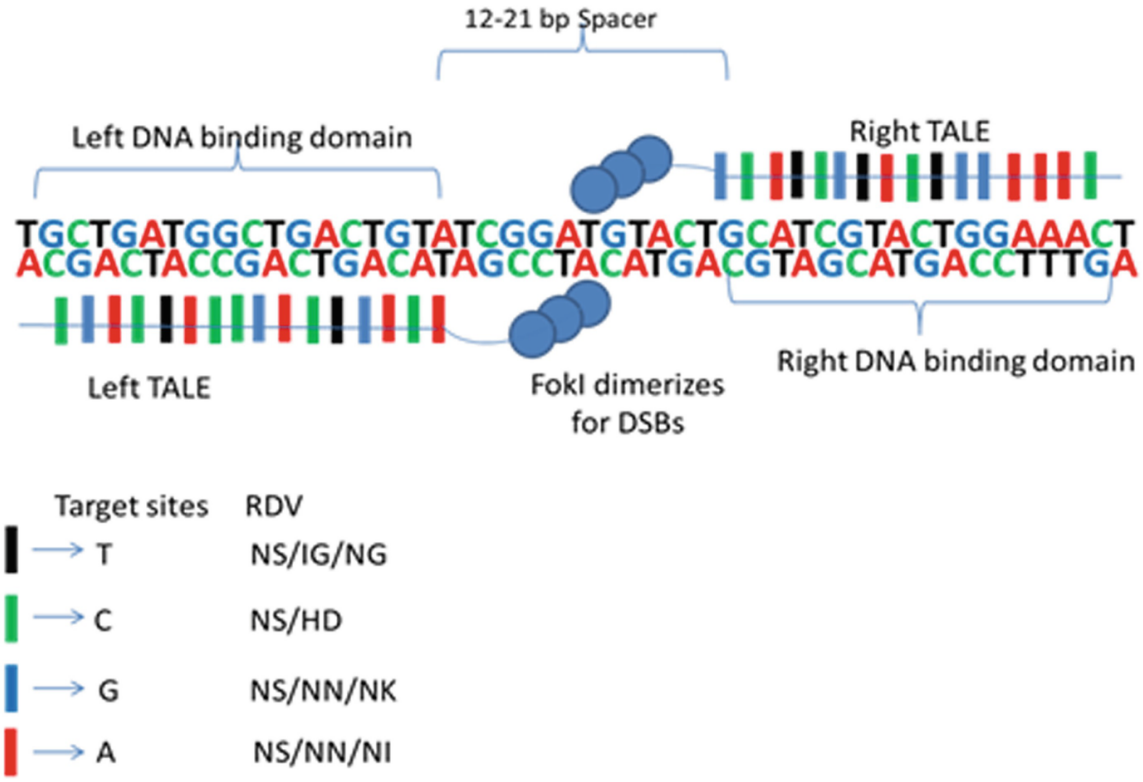


Fig. 5 Schematic illustration of TALEN constructs and its function. (Collected from our previous article Sen et al. 2023a)

6.3 ZFN

Zinc Finger Proteins (ZFPs) in the modern era of biotechnology has broadened the chance of crop improvement in terms of resilience, adaptation, quality parameters and yield. In plant biotechnology, ZFPs have been employed as either zinc finger nucleases (ZFNs) or zinc finger protein transcription factors (ZFP-TFs). Zinc finger proteins (ZFPs) have a modular structure that provides an alluring framework for creating ZFNs. In most cases, these methods utilize site-directed nucleases (SDNs) that consist of DNA-binding domains (ZFPs) connected to a nuclease domain. A particular 3-bp DNA sequence is recognized by each designed zinc finger (ZF) with conjugated Cys2His2 motifs based on the residues in α helix. To recognize a particular DNA sequence (12–18 bp), four to six separate ZFs are often coupled together. Two ZF proteins are needed to target the appropriate DNA sequence (24–36 nucleotides), as the FokI nuclease works as a dimer to cleave double-strand DNA. The DNA-binding domain ensures specificity to particular DNA sequences, while the nuclease domain induces

double-stranded DNA breaks at the intended sequence. The host repair pathways, such as nonhomologous end joining (NHEJ) and homology-directed repair (HDR), are responsible for repairing the double-stranded breaks (DSBs) caused by these reagents. ZFNs have been extensively used for genome editing of different crops in last two decades (Petolino 2015). In case of plants ZFN was first used in Arabidopsis with 0.2% mutation rate and 10% transmission rate in the next generation (Lloyd et al. 2005).

After identifying the target gene for silencing, a ZFPs construct is designed, followed by constructing ZFNs. Subsequently, transformation is carried out using plasmids, and the T0 generation is cultured. Positive seedlings are selected for transgenic development and phenotypic studies. Notable target genome-editing tools for ZFNs include ZFNGenome v2.0, ZifBASE, Zinc-Finger Database (ZiFDB), and Zinc-Finger Tool, EENdb (Fig. 6).

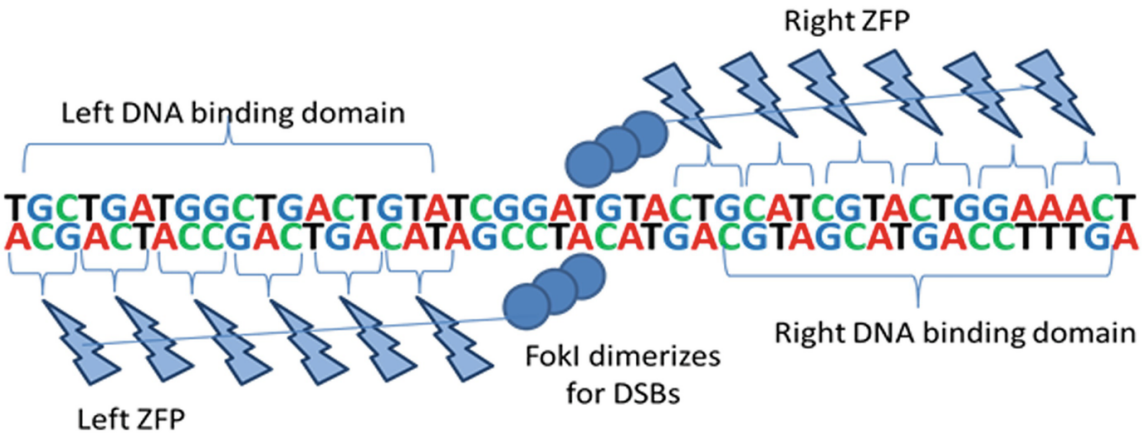


Fig. 6 Anatomy of typical ZFP constructs. (Collected from our previous article Sen et al. 2023b)

Up to date research on legumes for biofortification with the aids of genome editing techniques has been depicted in Table 4.

Table 4 The gene editing work in legumes for nutritional quality improvement

Crop	Technique	Gene name	Character	References
Glycine max	CRISPR/Cas9	GmFAD2–1A and GmFAD2–1B	High oleic, low linoleic and α-linolenic acid	Do et al. (2019)
	CRISPR/Cas9	FAD2–2	Improved seed oil profile	al Amin et al. (2019)
	CRISPR/Cas9	GmF3H1, GmF3H2 and GmFNSII-1	Isoflavone	Zhang et al. (2020)
	TALEN	FAD2-1A, FAD2-1B	Increase in oleic acid	Haun et al. (2014)

Crop	Technique	Gene name	Character	References
			content, decrease in linolenic acid content	
	TALEN	FAD3A	Decrease in linolenic acid content	Demorest et al. (2016)
	CRISPR/Cas9 & TALEN	GmPDS11 and GmPDS18	Carotenoid	Du et al. (2016)
	CRISPR/Cas9	Glutamine synthase (GS1)	2 bp deletion	Michno et al. (2015)
	CRISPR/Cas9	Chalcone-flavanone isomerase (CHI20)	1–32 bp deletions	Michno et al. (2015)
Arachis hypogaea L.	TALEN	FAD2-1A, FAD2-1B	Increase in oleic acid content, decrease in linolenic acid content	Wen et al. (2018), Chi et al. (2011), Yuan et al. (2019)
Medicago truncatula	CRISPR/Cas9	MtPDS	Carotenoid	Meng et al. (2017)

There aren't many thorough examples in the literature right now that explain how genome editing can be used to biofortify pulses. Nevertheless, using genome editing methods to improve iron and zinc biofortification holds great promise. Many genes, including iron-regulated transporter (IRT), ferric-chelate reductase oxidase (FRO), YELLOW STRIPE 1-like (YSL), natural resistance-associated macrophage protein (NRAMP), zinc-regulated transporters, and zinc-regulated transporters like protein (ZIP), can be altered in order to accomplish this. By targeting these genes, it is possible to augment the uptake of iron and zinc in all pulse crops, thus contributing to their nutritional value (Tan et al. 2017). Still there are some examples of legumes like lentil, pea, common bean, faba bean, mung bean, where there is no new report of research on genome editing.

6.4 Regulatory Framework

Public and private breeders share the view that gene editing, the most recent advancement in genetic engineering, holds significant promise for the creation of new plant varieties. This innovation offers various avenues for genome modification, such as allele replacement, targeted deletions, targeted insertions (also known as site-directed nucleases SDN-1/2/3), and base conversion techniques (Friedrichs et al. 2019;

Marzec and Hensel 2020). The regulatory approach for each of these fields like agriculture, medicine, industry etc. may vary depending on the jurisdiction. Plant breeders may encounter diverse requisites for research, legal considerations, regulatory compliance, and marketing strategies when developing new gene-edited varieties utilizing these genome-editing techniques (Entine et al. 2021). Presently, all plant varieties generated through gene-editing technology must follow to the same standards as genetically modified organisms (GMOs) as mandated by the European Union (EU). In October 2016, the Office of the Gene Technology Regulator (OGTR) in Australia initiated a Technical Review of Gene Regulations, leading to proposed changes. Under these new regulations, genetically modified organism (GMO) regulations would not be applicable to organisms modified using site-directed nucleases without templates for genome repair, also known as SDN-1. In Canada, biotechnology products derived from gene editing are subject to pre-market safety assessments only if they are deemed new and could potentially pose novel risks. However, gene-edited products do not require pre-market safety assessments in Canada if they do not display a novel trait. Argentina was among the pioneers in adopting regulatory measures for new (plant) breeding techniques (N(P)BTs) in 2015, encompassing genome-editing as a subset. If the NBT does not introduce new genetic material, it is classified under non-genetically modified (non-GM) regulatory frameworks. Due to country-specific regulations, global harmonization of genome-editing regulations is not currently feasible. Different jurisdictions employ a variety of regulatory and policy approaches to genome editing, necessitating an understanding of these differences. While uniform global approaches may not be achievable, establishing a common understanding is crucial for mitigating challenges stemming from varying regulations. For genome editing to gain traction as a marketable technology, all stakeholders must prioritize effective communication and information exchange. Advocates and opponents alike should transparently communicate risks grounded in scientific evidence, avoiding sensationalism or fear-mongering tactics that may mislead the public. This approach fosters a more informed discourse and enables individuals to make decisions based on accurate information.

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7 Conclusions and Future Prospects

There is enormous opportunity to improve the nutritional content of legume crops through the production of biofortified cultivars using gene editing and genetic modifications. Leveraging the natural variability in iron (Fe), zinc (Zn), and other micronutrient content in food legumes

offers opportunities to increase their levels and improve bioavailability. Addressing anti-nutritional factors such as phytate, trypsin, and chymotrypsin inhibitors can also improve the bioavailability and intake of legumes. Identifying genotypes with low anti-nutrient content in germplasm, induced mutagenesis, or genome editing are key objectives in legume improvement programs aimed at obtaining nutritional security. Additionally, crop wild relatives of various legumes, which lack these anti-nutrients, could be utilized in pre-breeding programs to further enhance nutritional quality. Much of the research is currently focused on gene characterization at the laboratory scale, which poses a significant constraint on the effective marketing of legumes. Ensuring the stability and transfer of transgenic expression throughout generations requires thorough evaluation under field conditions. Additionally, it is apparent that the success of transgenic biology in legumes is heavily influenced by genotype variability. Exploring alternative transformation system that does not rely on tissue culture, as well as manipulating morphogenic regulators, presents potential solutions to overcome these challenges.

While CRISPR technology is still gaining traction for regular use in legumes, it is evident that this next-generation genome-editing tool will play a significant role in legume breeding studies. Its adoption is expected to enhance productivity and improve biotic and abiotic stress tolerance. Key advancements such as improving technical capabilities (e.g., regeneration and transformation), reducing off-target effects, developing multiple PAM site selection systems, creating non tissue-culture based protocols, enhancing homology-directed repair (HDR) and viral vector efficiencies for CRISPR, and addressing regulatory and policy challenges will further facilitate the integration of gene editing technology into legume breeding programs. Nonetheless, the successful use of genome editing for legume enhancement will be dependent on the availability of proficient methods for plant transformation and full plant regeneration, as well as a supportive regulatory environment and public acceptance of gene-edited crops.

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Enhancing Shelf Life and Nutritional Quality of Leguminous Microgreens: Insights into the Application of Nanotechnology, Hydroponics, and Genetic Engineering Approaches

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Abbreviations

B Boron
Bp base pair
Ca Calcium
Cas9 CRISPR-associated protein 9
CDEN Chitosan-dazomet nanoparticles
CHDE Chitosan-hexaconazole-dazomet nanoparticles
CHEN Chitosan hexaconazole nanoparticles
CO_2 Carbon Dioxide
CRISPR Clustered regularly interspaced short palindromic repeat
Cu Copper
CuO Copper oxide
DNA Deoxyribonucleic acid
Fe_2O_3 Ferric oxide
Fe_3O_4 Ferrous oxide
Fe Iron
FRET Fluorescence resonance energy transfer

GIS Geographic Information System
GMO Genetically modified organisms
GPS Global Positioning System
Kg Kilogram
K Potassium
L Litre
Mg Magnesium
Ml milliliter
Mn Manganese
Mo Molybdenum
NAA 1-naphthalene acetic acid
NFT Nutrient film technique system
NGS Next generation sequencing
nm nanometer
N Nitrogen
NPs Nanoparticles
P Phosphorus
ppm part per million
Se Selenium
SiO_2 Silicon dioxide
SNP Single nucleotide polymorphism
S Sulfur
SWNT Single-walled carbon nanotubes
TALEN Transcription activator-like effector nucleases
TiO_2 Titanium dioxide
ZFN Zinc finger nucleases
ZnO Zinc oxide
Zn Zinc
2,4-D 2,4-dichlorophenoxyacetic acid
µg Microgram
µM microMolar

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1 Introduction

Our daily diet holds great significance not only in providing sufficient energy to sustain and survive but also in providing essential nutrition and thus preventing human health problems arising from nutritional

deficiencies (Choe et al. 2018). With this regard, the food industry has now started to focus on healthier food with low calorie and fat, high pre/probiotics, and antioxidant activities. This awareness of high-nutrition and health-focused food has given a boost to emerging new foods such as microgreens in the agricultural industry. Microgreens are typically young vegetable seedlings, harvested within 10–20 days of germination (Verlinden 2020). Some of the commonly grown microgreens include lettuce, mustard, cabbage, radish, pea tendrils, etc. They are richer in minerals, vitamins, phytoactive compounds, and antioxidants in comparison to their mature selves (Mir et al. 2017). For long, chefs at high-end restaurants have been using various microgreens in soups, salads, sandwiches, and other culinary items primarily for garnishing (Teng et al. 2021). Due to their enhanced quality traits, microgreens have become even more important and have extended their uses towards the enrichment of diet according to consumers' demands. They become highly important food for vegans, a sect of people who rely specifically on nutrient-rich raw dietary foods. Besides the nutritional benefits that microgreens provide, their short production cycle and little space occupancy make them highly suitable future food. As the world population is constantly increasing, microgreens are attributed as a portion of potential functional food for combating diseases caused by malnutrition, obesity, and lifestyle-related diseases like diabetes (Bhaswant et al. 2023). While microgreens impart a wide range of benefits over many other food alternatives, their production and sustainability lack in meeting the increasing demands of such a huge consumer population. Their production technologies, maintenance, pre- and post-harvesting techniques, shelf life, and packaging are a few major steps that require special attention. In order to optimize post-harvest maintenance, increasing shelf life, and large-scale production, various techniques have been employed in the micro-scale vegetable production industries (Mir et al. 2017). Many studies have highlighted various standardized procedures of hydroponics towards obtaining high-quality microgreens due to the advantage that it provides for controlling environmental conditions such as water and air temperature, nutritional solution, photoperiod, humidity, etc. (Moraru et al. 2022). Furthermore, advancements in biotechnology have given birth to a whole new field, nanotechnology where nanomaterials enable the controlled release of nutrients and site-specific delivery, and have also been helpful in disease management, these are some of the many applications provided by nanotechnology in combination with hydroponics (Maluin et al. 2021; Teng et al. 2023). Moreover, transformation and genetic approaches have also been developed to change the genetic makeup to achieve enhanced desired bioactive compound synthesis in cells. These approaches have been used by many researchers to obtain some nutritionally enriched species such as *Brassica* spp. (Zuluaga et al. 2019), *Oryza sativa* (Paul et al. 2012), *Nicotiana benthamiana* (Morelli et al. 2023), chickpeas (Mahto et al. 2022),

and pigeon pea (Arya et al. 2022). Researchers make use of different techniques, discussed in detail later in this chapter, for the successful integration of desired genetic material, suitable for employing improved traits in microgreens.

Although microgreens make potential superfoods for future generations, some factors need special attention and are required to be addressed properly to make this superfood available to meet the increasing demand. This chapter discusses the various techniques and procedures for enhanced production and improved shelf life of microgreens with a focused lens on leguminous microgreens.

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2 Nanotechnology: A Molecular Approach to Precision Agriculture

Nanotechnology deals with bringing advancements using nanomaterials, which are named after their nanoscale size. Nanomaterials are 100 nm in size in at least one dimension, owing to their high surface-to-volume ratio, they have gained huge popularity in the scientific world. This concept was first introduced by Nobel laureate Richard P. Feynman, the title of his lectures in 1959 said “There’s Plenty of Room at the Bottom: An Invitation to Enter a New Field of Physics.”. However, applications of nanotechnology could not be restricted to physics, its usage in biological sciences began no earlier than the 1980s giving birth to a whole new field of biology later called Nanobiotechnology (El-Chaghaby and Rashad 2023). Scientists have introduced two approaches for enabling the fabrication of nanomaterials: the top-down approach, wherein bulk material is fabricated to form nano-scale material having the same properties as the starting material, and the bottom-up approach, where small molecules are coagulated such as to form nano-scale material. Nanomaterials are produced in different shapes and sizes depending on their application, there can be 3 methods of nanomaterial synthesis viz. physical, chemical, and biological or green synthesis. Those produced biologically using plant-based material, also called green nanoparticles, are most desirable as they keep in check the toxicity that might be caused by excess usage of nanoparticles (Haris et al. 2023). This field of technology gained huge popularity in medicine and engineering, the versatility of its applications in different fields has led nanotechnology to find its space in all kinds of industries such as computers, cosmetics, bioprocessing, healthcare, food, packaging, and agri-industries to name a few (Malik et al. 2023).

Different agricultural practices have gained benefits from nanotechnologies. In broad terms, nanomaterials have enabled efficient usage of fertilizers resulting in better yield; reduced pesticide usage due to smart delivery systems; early detection of harmful chemicals in food items; and last but not the least, better system for packaging and processing of

food items. Conventional fertilizers and pesticide application methods lead to huge losses due to soil leaching, drifting, and runoffs, causing 50–70% of chemicals to be leached into the soil. Farmers are required to make repetitive applications of these chemicals to enable the required amount of nutrients to enter the plant. This leads to natural soil resources degradation, soil microflora getting affected, and pests and pathogens becoming resistant due to continuous exposure and accumulation of pesticides (Usman et al. 2020).

Furthermore, the structure and surface chemistry of nanomaterials can be modified to offer controlled delivery strategies and to provide the opportunity to direct the right location and functionality within the plant. Utilizing these factors, some studies have used nanomaterials for improving various traits in leguminous microgreens such as Ferric oxide (Fe_2O_3) nanoparticle application has resulted in better growth, biomass, and iron fortification in microgreen plants. Similarly, improved root and shoot length, metabolites, and enzymes in tobacco microgreens were observed when treated with a limited concentration of ZnO nanoparticles. Selenium nanoparticle treatment in tomato microgreens has helped the plant cope with environmental stresses. In Soybean microgreens, silver nanoparticles helped in mitigating metal toxicity. Researchers have also worked toward nanoparticles applications for improving the shelf life of microgreens, one such study uses straw nanofiber to improve the tissue and shelf life of green beans (*Phaseolus vulgaris*) (Aloo et al. 2021). Furthermore, studies that have used nanomaterials for crop enhancement are listed in Table 1.

Table 1 Crop plants treated with nanoparticles to study their enhanced physiological factors

Crop	Nanoparticle	Optimum concentration	Factors affected	Medium of cultivation	Time of Harvest	References
Spinach (*Spinacea oleracea* L.)	Fe_2O_3 (30–40 nm)	100–200 mg	Boost growth rate	Hydroponic (Hoagland nutrient solution)	45 days	Jeyasubramanian et al. (2016)
Tobacco (*Nicotiana tabacum* L.)	ZnO (25 nm)	0.2–5 µg	Increase in biomass, amino acid content, phenols, proline, antioxidants	Hydroponic (Johnson nutrient solution)	21 days	Tirani et al. (2019)
Tomato (*Solanum lycopersicum* L.)	Se-nanoparticles (8–15 nm)	4–12 µM	Increase in fresh and dry weight of shoots and roots, and	Hydroponic solution mixture containing N, Mo, P, K, Ca, B,	–	Haghighi et al. (2014)

Crop	Nanoparticle	Optimum concentration	Factors affected	Medium of cultivation	Time of Harvest	References
			chlorophyll content	Mg, S, Fe, Mn, Zn, Cu		
Maize (*Zea mays* L.)	SiO_2 (20–40 nm)	1%	Increase in germination and dry weight	Hydroponic (Hoagland nutrient solution)	15 days	Suriyaprabha et al. (2012)
Tomato (*Solanum lycopersicum* L.)	Hydroxypatite (94–163 nm)	200, 500 mg/L	Increase in root length	Hydroponic (Hoagland nutrient solution)	30 days	Marchiol et al. (2019)
Tomato (*Solanum lycopersicum* L.)	Fe_3O4, TiO_2 (10–30 nm)	50, 500 mg/L	Increased root hair	Hydroponic solution mixture of N, P, Ca, K, S, Fe, Zn, Mn, Cu	17 days	Giordani et al. (2012)
Cucumber (*Cucumis sativus* L.)	Cu-Fe_2O_4	0.04–5.00 ppm	Increased fresh weight, protein content, antioxidants, absorption of Fe and Cu	Hydroponic (Hoagland nutrient solution)	45 days	Abu-Elsaad and Hameed (2019)

2.1 Nanoscale Delivery Systems for Targeted Nutrient Delivery

To maintain sustenance of food production and food security, nanotechnology has made remarkable achievements in finding applications in improving pre- and post-food production techniques, starting from controlled delivery of nanopesticides, fertilizers, and herbicides for improved yield to nanosensors required to monitor environmental conditions including biotic and abiotic stresses. Precision farming techniques are employed for enhanced nutrient and fertilizer uptake with the application of high adsorption capacity nanomaterials such as nanoclays and zeolites which are increasingly being used for smart nutrient delivery. Nanomaterials have small sizes and large surface areas; they are even tinier than pores in the roots and leaves of the plant and are designed to match the uptake patterns of molecules in crops to allow them to cross biological barriers and provide more accurate nutrient delivery. Metastability of some nanomaterials has also helped better micronutrients and antibiotics delivery in plants resulting in better management of diseases and crop losses (Sharma et al. 2022).

Nano-fertilizers provide various advantages over ordinary fertilizers as many studies have reported harmful effects for humans if the latter is used in excess, while in former, fertilizers are encapsulated to not only protect

the active ingredient but the surface coating of biocompatible polymers also enable the slow and controlled release in plants, preventing loss of excessive fertilizers and providing maximum availability to the plant (Sharma et al. 2022).

2.2 Nanosensors for Real-Time Monitoring in Microgreen Cultivation

Nanotechnologies have made revolutionary advancements in electronics in the last several decades such as the advancements in Global Positioning System (GPS) and Geographic Information System (GIS). Nano-scaled sensors and monitors which when combined with agronomic, environmental, climatic, and other relevant data provide more precise monitoring of the factors contributing to the healthy survival of the crop. Nano-scaled sensors or Nano-sensors can detect and quantify physical and chemical factors at a very small scale in both industries and biological systems and provide real-time monitoring in microgreen cultivation. They have recently been employed for the detection of heavy metal accumulation, plant diseases, chemical composition of the growing medium, optimum temperature, required nutrient concentration, pH, etc. Moreover, real-time assessment of the quality of soil, other plant-growing mediums like aquaponic and hydroponic solutions, and the presence of disease-causing organisms can also be done using nanosensors connected to the Global Positioning System (GPS) (El-Chaghaby and Rashad 2023).

2.3 Applications of Nanotechnology in Quality Enhancement and Improvement of Shelf Life of Microgreens

The nano-sensor technologies provide real-time detection tools for pests, pathogens, microbes, contaminants, and other toxic substances. Some of the nanosensors currently in use are carbon-based nanotubes, electrochemical biosensors, nanoplasmonic, nanowires, antibody nanosensors, and FRET-based nanosensors (Sharma et al. 2023a). Chitosan-based nanoparticles are popularly used in different fields due to their non-toxic, renewable, biocompatible, and biodegradable traits. Examples include Chitosan hexaconazole nanoparticles (CHEN), chitosan-hexaconazole-dazomet nanoparticles (CHDE), and chitosan-dazomet nanoparticles (CDEN), their antifungal and antimicrobial activity is exploited in synthesizing packaging material for harvested microgreens (Sharma et al. 2023a). Microgreens quality and durability can be improved using nano-based packaging material by restricting physiological maturity, microbial invasion, controlling water loss, gaseous exchange, quality deterioration, and solvent discharge in packed microgreens (Sharma et al. 2023a). Nano coating-based packaging is one of the most exploited approaches for improved food packaging. Examples of various nano-coatings are protein isolate coatings, chitosan nanocomposite film coating, galvanization of chitosan on silicon nanoparticle/titanium oxide

nanoparticle, nitric oxide coating chitosan nanoparticle, edible nanolaminate coatings synthesized using *Flourensia cernua* extract, alginate containing nano-emulsified basil oil, edible nano-alginate and limonene, etc. (Sharma et al. 2023a). Fortification of microgreens is achieved by highly stable nanoparticles synthesized using essential nutrients like Cu, Se, Fe, and Zn. These are increasingly being utilized for the improvement of microgreen germination, nutrition, and seedling growth (Gupta et al. 2023). Nanoparticles-mediated genetic transformation is a novel approach developed for the accurate delivery of genetic material. It is usually combined with other transformation methods which may include biolistic particles, electroporation, and *Agrobacterium* to achieve stable transformation (Lv et al. 2020).

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3 Hydroponics: Cultivating Microgreens Beyond Soil

Soil has been the major medium for cultivating plants for ages, traditional agriculture farming (Open field agriculture) depends solely on soil as it provides proper space, nutrients, and enough water. Despite being the first choice for agriculture, some cons such as degradation due to soil erosion, the occurrence of bacteria and nematodes resulting in soil-borne diseases, and soil infertility are arising in conventional soil agriculture. Additionally, conventional agriculture requires large land space, large amounts of water, and a lot of manpower for laborious fieldwork. Moreover, the continuously expanding population is leading to rapid industrialization and development in metropolitan cities hence increasing a threat to food security due to a reduction in agricultural land and soil fertility. Also, in some distant areas, due to unfavourable geographical and topological conditions, there is limited availability of fertile and arable land. Therefore, the soil-less culture techniques might come up as a boon for the future of agriculture. Soil-less agriculture helps us to face the challenges caused by climate change, reduction in fertile land and to cope with the continuously increasing food demand. In soil-less agricultural techniques such as hydroponics, aeroponics, and aquaponics, the plants are grown in a closed and controlled environment with well-planned utilization of natural resources. (Butler and Oebker 1962).

Hydroponics is an old technique that has gained attention only recently. In hydroponics, the nutrient-rich solution is provided to the plant in such a manner that plants harbour in ideal nutritional conditions taking care of space constraints, reducing water losses, and enabling recycling of nutrient solutions. The hanging gardens of Babylon in earlier times worked on the principle of hydroponics. This technique has been very popular in Europe since earlier times, the biggest market being Spain, Netherlands, and France. Some Asian countries are also expanding hydroponic crop

production now. In India, hydroponics was first introduced by an English scientist W.J. Shalto Duglas in 1946. (Hughes 2017). The word Hydroponics comprises two Greek words ‘Hydro’ means water and ‘pronos’ means labour. It is an innovative and popular technique for growing plants and vegetables with their roots immersed in a nutrient-rich aqueous solution in a soilless manner (Beibel 1958). It has been utilized for research purposes for years, it has now entered the commercial crop production market as well.

Hydroponics technique is well utilized to produce less space-consuming small crop systems such as microgreens which are highly rich in nutrition and easy to grow through hydroponics (Rajan et al. 2019). The growth of microgreens is faster in hydroponic solution as compared to soil beds as it support many crop cycles per year thereby increasing their per-year yield. Microgreens are being cultivated in different types of mediums for better growth, a few of them are mentioned in Table 2.

Table 2 List of different studies on microgreen crops with their medium of cultivation

Family	Microgreens	Medium of cultivation	References
Brassicaceae	*Eruca vesicaria* L. Cav. subsp. *Sativa*	Rockwool under greenhouse	Murphy and Pill (2010)
	Brassica oleracea var. *capitata*	Soil-based under greenhouse	Sun et al. (2013)
	Brassica oleracea L. var. *capitata*	Soil-based under greenhouse	Kamal et al. (2020)
	Rapanus sativus L.	Hydroponic systems	Xiao et al. (2019)
	Rapanus sativus L.	Rockwool, white sphagnum peat, Coco coir	Mezeyova et al. (2022)
	Brassica rapa L.	Rockwool under green house	Mezeyova et al. (2022)
	Raphanus sativus L. var. lomgipinnatus	Hydroponics system	Xiao et al. (2019)
	Brassica narinosa L. var. rosularis	Soil and mat cultivation under a growth chamber	Kamal et al. (2020)
	Brassica rapa L. var. rapa	Soil and mat cultivation under a growth chamber	Kamal et al. (2020)
Lamiaceae	*Ocimum basilicum L.*	Rockwool, vermiculite, cotton and jute fiber	Bulgari et al. (2017), Bulgari et al. (2021), Mezeyova et al. (2022)
Amaranthaceae	*Beta vulgaris subsp. vulgaris*	Soil and mat cultivation	Lee et al. (2004)
	Beta vulgaris subsp. vulgaris	Soil-based	Ramya et al. (2020)
	Amaranthus tricolor	Soil-based under greenhouse	Ebert et al. (2015)

Family	Microgreens	Medium of cultivation	References
Cucurbitaceae	*Cucumis sativus*	Soil-based	Ramya et al. (2020)
Asteraceae	*Lactuca sativa*	Soil-based	Pinto and Ferreira (2015)
	Lactuca sativa	Sphagnum moss	Ramya et al. (2020)
Chenopodiaceae	*Spinacia oleracea L.*	Soil-based cultivation	Xiao et al. (2019)
Polygonaceae	*Rumex acetosa L.*	Soil-based cultivation	Xiao et al. (2019)
Basellaceae	*Basella alba*	Soil and mat cultivation under a growth chamber	Muchjajib et al. (2014)
Poaceae	*Zea mays L.*	Soil-based cultivation	Xiao et al. (2019)
Leguminaceae	*Trigonella foenumgraecum*	Cocopeat	Ramya et al. (2020)
	Vigna radiata L.	Soil-based cultivation	Ramya et al. (2020)

3.1 Advantages of Hydroponics Over Traditional Soil-Based Farming

Hydroponics is a modern approach to ensure the large-scale production of many crops without soil in a small space (Muller et al. 2017). The hydroponic system shows 30–50% higher plant growth rates than soil-based cultivation systems, a study proved that hydroponically grown lettuce shows an 11 times higher growth rate and 10% less land use (Barbosa et al. 2015). Microgreen cultivation has shifted towards a modern hydroponic approach as it has proven to enhance and accelerate plant growth, help in higher yield production, crops are easier to harvest and it is unaffected by changing seasons and other external environmental factors.

Leguminous microgreen cultivation can be combined with a vertical farming system where land area usage for agriculture can be drastically reduced. Such types of agricultural practices are the future of compact metropolitan cities. This paves the way to produce high-value nutritional food in limited areas available.

In traditional soil-based cultivation, plants tend to compete for space and nutrients, while hydroponic cultures are made nutritionally rich according to the requirements of crop plants. Another advantage that hydroponics provides over soil-based farming is the controlled and regulated environmental conditions, as a result, hydroponic cultures hardly fall prey to natural calamities and sudden environmental stresses. This helps to prevent excessive losses occurring due to sudden weather changes. The plants grown hydroponically in a greenhouse are comparatively less environmentally impacted as compared to plants grown traditionally in the greenhouse due to extensive usage of pesticides and fertilizers in the latter case (Russo and Scarascia Mugnozza 2004). Chemical-free cultivation of microgreens makes the hydroponic system

more suitable for the environment and healthy for the consumers. Table 3 summarizes the advantages and disadvantages of different substrates of microgreen cultivation.

Table 3 Pros and Cons of different types of substrates used for microgreen cultivation

Substrate for cultivation	Advantages	Disadvantages
Soil	Provide humus	Microbial contamination
Solid substrate other than soil (peat, jute, textile fibres)	High pore space, high water retention, cheap	High salt concertation, non-renewable
Hydroponics	Nutrient solutions can be reused or recycled, suitable for vertical farming, season-independent	Microbial internalization
Hydrogel	Gradual release of nutrient solution, low microbial infestation, reusable	Easily dehydrate slow absorption of nutrient solution.

Adopted from Du et al. (2022)

Wastewater reuse is another advantage of the hydroponic system, treated and domestic wastewater is used as a nutrient medium for growing plants (Sutar et al. 2018). A study on cucumbers and tomatoes, grown hydroponically, showed that they can be grown under 33% of drainage water (Grewal et al. 2011). Whereas in the case of soil-based agriculture, water re-utilization requires adequate approaches (Egbuikwem et al. 2020).

A study on microgreens shows that Biochemical content was found to be higher in the case of soilless culture as compared to soil-based culture. Various microgreen crops like violet basil, lettuce, radish, dill, green basil, and coriander were studied which showed higher micronutrient content (magnesium, iron, zinc, copper), phenolic compounds, and antioxidants with good morphology if grown on soilless culture as compared to soil-grown plants (Poorshahabad et al. 2019).

Different types of Hydroponics systems are used to grow microgreens **(**Luz et al. 2008; Okemwa 2015; Nguyen et al. 2016; Khan et al. 2023).

3.1.1 Deep Water Culture System

It is the most common and popular type of hydroponic method. In this method, plants are held by pots that are suspended in an oxygen-rich nutrient-rich solution. Their roots are immersed in a solution, which helps in the uptake of nutrients, water, and oxygen. An air stone is placed at the bottom of the reservoir and connected to an air pump which takes care of the proper oxygenation of the entire system. Here the water is circulated continuously to ensure the proper availability of nutrients to the plant roots. This system needs a constant supply of electricity to run the air pump. This system is easy to set up and maintain. However, a continuous

supply of nutrients is a must otherwise plants may not receive optimal nutrients or may become overwatered.

3.1.2 Nutrient Film Technique System (NFT)

In this type of system, plant roots are submerged in a continuous stream of nutrient solution, which is supported by sloping channels. The water is circulated continuously to replenish the optimal nutrient requirement of plants and is also connected to air stone for efficient aeration. The nutrient solution is continuously pumped through channels by a submersible pump.

3.1.2.1 Ebb and Flow System

This system also known as a flood and drain system, comprises of grow tray and a reservoir. Here a pump is used which periodically pumps nutrient-rich water solution to the growing area which is then slowly drained back. This system is easy to set up and maintain and perfect for efficient water usage. However, the timing of flooding and draining is crucial otherwise leading to uneven watering of plants. This system is also prone to root rot, mold, and algae.

3.1.2.2 Drip System

In this type of hydroponic system, the plants are placed separately from the nutrient-rich reservoir. The nutrient-rich solution is delivered to the plant through a series of tubes and nozzles in a controlled manner with the help of a water pump, placed at the bottom of the reservoir. The extra nutrient solution is drained out or recollected and circulated again through pumps. However, maintenance of emitters and nozzles is important otherwise if the emitters become blocked, it may be lethal to the plants.

3.1.2.3 Aeroponics

It is another type of hydroponic system that uses nutrient-rich water solution for growth. Here, plant roots are suspended in air and nutrient-rich water solution is misted onto the roots of the plants. A surplus amount of oxygen is provided which helps in the absorption of an efficient amount of nutrients. It is a very good technique as the misting of nutrient solution helps in a high rate of absorption. However, it is expensive to set and maintain, and nutrient solution must be misted properly otherwise leads to the overwatering of plants.

3.1.2.4 Wick System

In this type of hydroponic system, a soft fabric made of cotton or nylon called a wick is used to transport nutrient-rich water solution from a reservoir to plant roots. Plants are placed in a growing medium on a tray which is placed above a reservoir of nutrient solution. The nutrient solution flows with the help of a wick in capillary action from the reservoir to the plants. So, this technique does not require a pump and electricity to work.

The system is easy to set up and maintain. However, this system is only governed by capillary action hence the process is slow.

3.2 Environmental Factors to Be Examined for Microgreen Hydroponic Cultivation

Microgreens are generally harvested in 7–21 days from the day of sowing, during this period, it is important to maintain or control certain environmental factors for the efficient growth of microgreens. These factors include nutrient solution, light regime, pH, dissolved oxygen, relative humidity, air and water temperature, electrical conductivity, and carbon dioxide concentration (Fig. 1), which are crucial during the early phase of plant development which affects the final biofortification of microgreen crops (Akula and Ravishankar 2011; Lu et al. 2017).

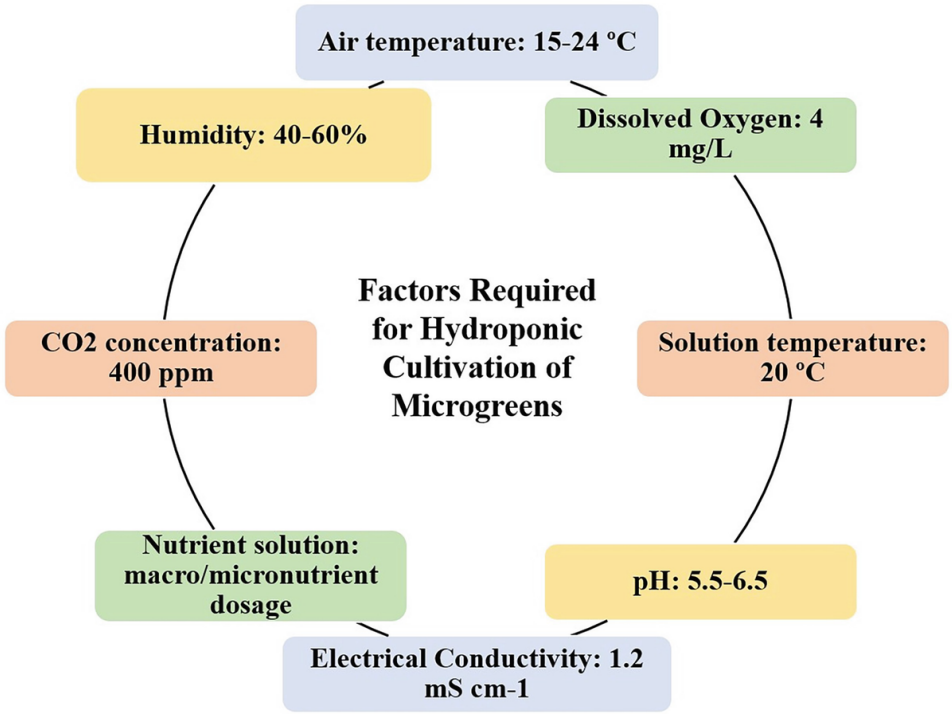


Fig. 1 Controlled environmental factors required for the maintenance of hydroponically grown microgreens

3.2.1 Nutrients

Nutrients in soilless culture are provided through organic fertilizers like Azomite for improving plant growth in soilless, substrate-based, and soil-based cultivation. Generally, microgreens are grown without fertilizers but sometimes nitrogen fertilizer at 50–150 ppm along with 20-10-20 straight fertilizers are used to enhance microgreens growth (Sharma et al. 2023b).

3.2.2 Temperature and Humidity

The optimum temperature for microgreens is 18–24 degrees with 40–60% humidity in open and closed soilless culture systems. For leguminous specifically beans microgreens, it is around 15–21 degrees with 40–50% humidity is required for optimal plant growth (Sharma et al. 2023b).

3.2.3 Light

Generally, microgreens can grow well in both artificial and natural light conditions. However, 12–18 hr of light period and 4–6 hr of dark period are optimal for microgreens growth in open and closed systems. The light period induces photosynthesis and food production whereas the dark period induces germination and stem elongation in microgreens. Abrupt light conditions affect the growth and flavor of microgreens. A light duration of 12–13 hr is optimum for the efficient growth of bean microgreens (Sharma et al. 2023b).

3.2.4 Water

A specific quantity of water is required for microgreens to grow. The pH and temperature of the water are around 5.5–6.5 (acidic to neutral) and 10–16 degrees respectively. Dechlorinated water should be used as high chlorine alters the color and flavor of microgreens (Sharma et al. 2023b).

3.2.5 pH

The optimal pH for microgreen production is 5.5–6.5 (Sharma et al. 2023b).

Successful implementation of hydroponically grown microgreens has been reported in several crops. Ciuta et al. (2020) performed a comparative study of two hydroponics systems, ebb and flow benches and a vertical hydroponic system with two substrates used in the culture i.e., Peat and perlite mixed with cellulose. They used four species of microgreen production (Red radish, red basil, Peas, and Sunflower) for the study. Parameters like Germination rate, plant height, and weight of microgreens were taken into consideration. They observed a high germination rate with peat and perlite as compared to cellulose substrate. Overall, they observed higher yield in the case of the vertical system with peat and perlite as a substrate as compared to the ebb and flow system with cellulose (Ciuta et al. 2020). Another study reported the effect of the use of Reverse osmosis wastewater on hydroponically grown microgreens. The results showed the successful implication of the reuse of wastewater for irrigating and raising hydroponic microgreens (Selvan and Salvadeeswaran 2021). The leguminous crops commonly used for growing microgreens include *Cicer arietinum, lens culinaris, Medicago sativa, Trifolium repens, Vigna angularis*, and *Vigna radiata*.

4 Genetic Engineering for Improvement of Microgreens

Different molecular techniques, also involving genetic manipulation methods, have been developed by researchers to create better crop varieties (Fig. 2). In the past century, random mutagenesis methods, which use chemical compounds and irradiation methods, have been in use in mutagenesis based breeding programs. These methods are still popular in inducing random mutations to achieve the desired trait variety but their application involves drawbacks like non-specificity and low efficiency (Mao et al. 2019). Therefore, these drawbacks has resulted in the development of more specific engineered endonucleases. Restriction enzymes such as the mega-nucleases are large fragments (>14 bp) of DNA, they provide high specificity due to large recognition sites; zinc finger nucleases (ZFNs) are engineered to target specific sequences with zinc finger DNA binding domain fused with Fok1 DNA cleavage domain; transcription activator-like effector nucleases (TALENs), are also fused with Fok1 endonuclease for carrying out cleavage on their target sites; and the most recently developed, clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein 9 (Cas9) (Lv et al. 2020; Mandip and Steer 2019). Discovered initially in the 1980s in *Escherichia coli* in 3′ end of alkaline phosphatase gene, containing patterns of repeats and spacers, CRISPRs came into the limelight not before Next generation sequencing technology (NGS). Due to NGS, it became possible to sequence many organisms at a low cost in very little time, computational data analysis of the sequencing results gave insights into the occurrence of CRISPR in bacteria and archaea (Mandip and Steer 2019). Bacteria and archaea incorporate a portion of invasive genetic material from viruses, phages, and plasmids into their CRISPR locus to develop an adaptive immune system to protect themselves. CRISPR cas9 system is naturally absent in eukaryotes. Key elements of CRISPR machinery consist of Cas9, crRNA, and tracrRNA, this complex collectively carries out the targeted cleavage of DNA fragments resulting in its exploitation for the generation of a more efficient and highly specific genome editing tool. This Nobel-winning genome editing technique is considered to be a breakthrough in the scientific field (Horodecka and Duchler 2021). Apart from the genetic engineering techniques, other molecular techniques to alter crop yield and quality such as gene pyramiding, RNA interference (RNAi), combined with bioinformatic approaches such as sequencing technologies, identification of SNPs (Single nucleotide polymorphic markers), and hence marker-assisted selection (MAS) can help researchers in the selection of genomic regions associated with nutrition and shelf life of microgreens. The Advancement in sequencing technologies has also paved the way for the exploration of multi-omics approaches which include transcriptomics, proteomics,

genomics, metabolomics, etc. Till date, the applicability of these omics techniques has been very limited in the microgreens improvement but their applications are being utilized in crop improvement at a very large scale by researchers all around the world. Integration of these studies makes a better use of these studies such as a transcriptomic and metabolomic combined analysis by Wu et al. (2023) showed the accumulation of flavones and flavonols in Ligusticum chuanxiong microgreens upon blue light exposure. Foliage of microgreens is a reservoir of phytochemicals with high bioactive properties. The accumulation of glucosinolates was studied in Brassica by Alrifai et al. (2021) using LC-HRMS/MS, a metabolomics approach. Quantification of such phytochemicals and other secondary metabolites in microgreens is important for the selection of varieties with better trait values. Foliage is the most important part to elevate the shelf life of microgreens, a major limiting factor is the soft tissue in the young leaves which leads to deterioration during storage and transportation. In 1994, one of the earliest GMOs developed by Calgene (Monsanto) focussed on the disruption in the biosynthesis of a fruit ripening hormone, ethylene. The transgenic tomato was called ‘Flavr savr’, an antisense strategy was utilized to inhibit a gene which encode for polygalaturonase enzyme known to be involved in cell wall softening (Sheehy et al. 1988). Since long, researchers have also been interested in longivity of ornamental flowers such as transgenic torenia (Aida et al. 1998) and Petunia (Cobb et al. 2002). Similar technologies can be employed in the improvement of shelf life of microgreens. Over-expression, down-regulation, suppression, and transformation of genes of interest for altering pathways related to our desired trait are actively being researched for biofortifcation and enhanced shelf life of microgreens.

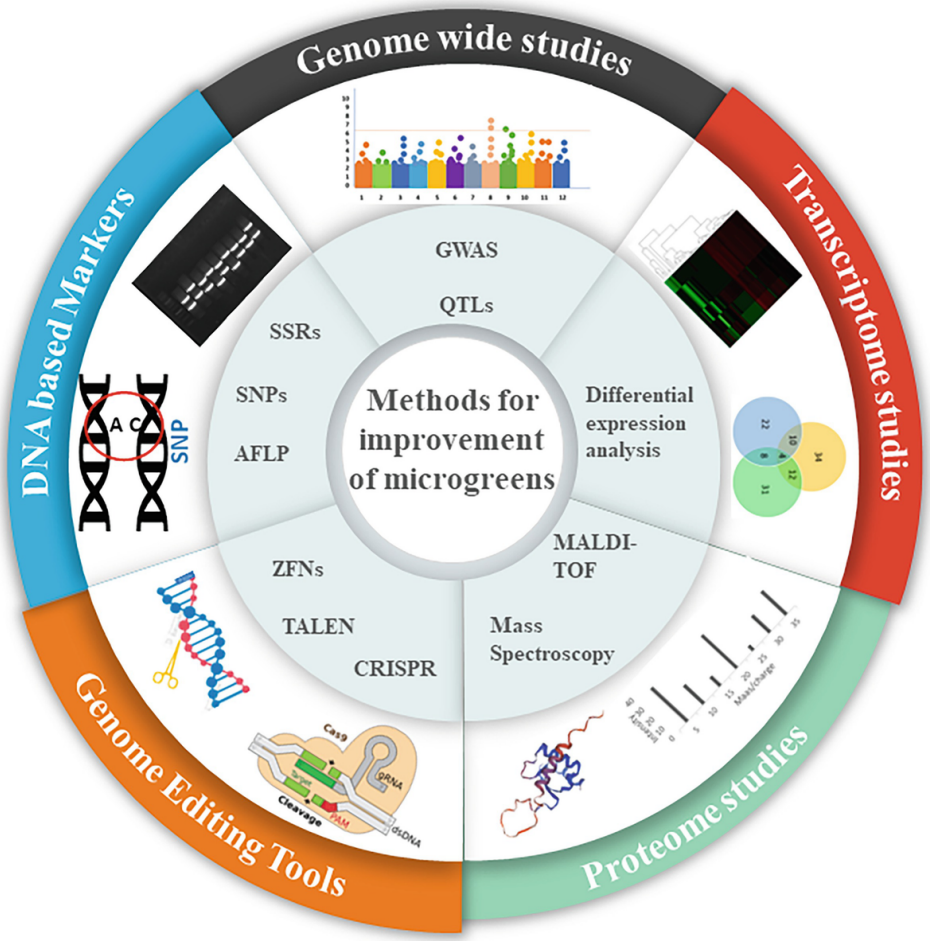


Fig. 2 An overview of different molecular techniques that can be used for the improvement of nutritional quality, quantity, and shelf life of microgreens

4.1 Genetic Enhancements for Nutritional Content and Disease Tolerance and Shelf Life Improvement of Microgreens

Genetic engineering approaches are extensively being utilized for the targeted modification of genes required for the development of varieties resistant to biotic and abiotic stress, increased productivity, and high nutritional value. Nutritionally improvised food is required to eradicate the problems of malnutrition all around the globe, notably in underdeveloped countries where poverty is a big issue. Some of the mind-blowing research has resulted in the alteration of metabolic pathways through genetic engineering to obtain high-nutritional crop varieties. Golden rice1 and Golden rice2 are characterized by increased beta-carotene content and a further increase in pro-vitamin A in the endosperm, respectively (Ye et al. 2000; Paine et al. 2005). A similar study in potatoes was done to obtain “Golden tubers” by altering the genes regulating the carotene synthesis pathway, genetically altered tubers looked deep yellow in color and showed

drastically increased levels of beta-carotene (Diretto et al. 2007). Furthermore, a significantly increased accumulation of metabolites was recorded in foliage along with tubers (Diretto et al. 2010; Nielsen et al. 2019). This enhancement of nutritional components particularly in foliage is of primary interest in the development of improved leguminous microgreen varieties.

Advancements in sequencing technologies have made it easier to identify and characterize genes involved in nutrient accumulation. Model leguminous plants, *Medicago truncatula* and *Lotus japonicus* have been sequenced to provide reference genomes to have an overall profile of the genes and enable the mining and transfer of desired traits to other species of the Fabaceae family. Other species like soybean, chickpea, and pegion pea have also been sequenced now, enabling comparative genome studies to uncover desirable candidate genes. Sequencing paves the way for the selection of genotypes using genomic marker-based approaches such as single nucleotide polymorphism (SNP). Further, the functional importance of potential candidate genes can be validated using genome editing techniques. Although genetic editing in legumes has not been as successful as in other species, the CRISPR/cas9 technique has been effectively implemented in cowpea, Soybean, and *Medicago trancatula* (Samal et al. 2023). Several transformation studies are reported in different leguminous plant systems, such as Agrobacterium-mediated transformation in Pisum sativum (Polowick et al. 2000; Shah et al. 2020), in *Cicer arietinum* (Gulati et al. 2002), Biolistics in *Lens culinaris* (Gulati et al. 2002), and Hairy root transformation in Soybean (Michno et al. 2015). Agrobacterium-mediated transformation led to the successful expression of OsNAS2 and CaNAS2 in chickpeas (*Cicer arietinum*) resulting in Fe biofortification of the crop (Das Bhowmik et al. 2019). Stable transgenic lines of biofortified leguminous microgreens can be developed using these techniques with appropriate genome editing tools. Various genome editing strategies are adopted in leguminous crops to address other important traits such as resistance to biotic and abiotic stresses. Biotic factors may include viruses, pathogens, and insects while abiotic factors are described by salinity, drought, flood, frost, and heat. The first stable transformation in legumes was carried out in *Vigna aconitifolia*, ever since, many such studies have progressed despite the struggles in transformation due to recalcitrance and genotype dependence of leguminous plants (Kohler et al. 1987; Choudhury and Rajam, 2021). Soybean became one of the earliest crops to be genetically modified and commercially available in the market, called Roundup Ready, in 1996 in the United States. It is also the only legume crop that is transformed using CRISPR/cas9 editing technology. More recently, attempts have been made to carry out smart breeding using genome editing for the improved shelf life of postharvest ornamental plants (Xu et al. 2020; Shibuya et al. 2018), fruits (Nishitani et al. 2016), and vegetables

(Hilioti et al. 2016). Microgreens, due to their high moisture content, undergo a 50% deterioration of their farm value while long-distance transportation. In the case of leguminous microgreens, integration of resistant genes can help extend their shelf life enabling improved storage and transportation of packed microgreens. Pathogens thriving on microgreens often become the major cause of their reduced shelf life, studies have been done to isolate them to get the whole genome sequence information which can be used further to identify microbial resistance genes (Partap et al. 2023). One such study by Naushad et al. (2022) have sequenced (MinION and MiSeq) three strains of Listeria monocytogenes collected from different microgreens. In another study, sequencing using Illumina NextSeq 500 led to the characterization of microgreen-associated bacterium species (Moon et al. 2022). The identified resistance genes will be used by researchers to develop resistant varieties of microgreens to combat such biotic stresses. Despite having several advantages, a major limitation of microgreens is their short shelf-life post-harvesting. They undergo dehydration, leaves start wilting as they are young and delicate, leading to early senescence. An inefficient storage system results in an all-time high cost of microgreens. Several attempts have been made to extend the shelf life of microgreens, in one study, pre-harvest treatment of microgreens with 10 mM of calcium chloride was found to delay the decline of quality and hence increase shelf life in broccoli microgreens (Kou et al. 2015).

4.2 Integration of Technologies: Synergies and Challenges

Agriculture is on the verge of revolution with a tremendous number of emerging technologies coming into the way of its development. People in earlier times have always worked towards better agricultural practices starting from working with hand tools to modern technologies taking over. New biological tools have managed to come up with solutions that can directly act on the plant systems for the generation of enhanced varieties. Above-described techniques are becoming acceleratingly popular among scientists for their potential of providing direct benefits to crops. The best output from new emerging technologies can be obtained through the integration of multiple disciplines that can complement each other.

The ability of nanoparticles to make a controlled and targeted delivery of nutrients has largely been exploited, even in hydroponic systems. In addition, nano biosensors have the potential to be used to detect pH, temperature, and nutrient status in hydroponic solutions (Chadwick et al. 2023). Plants absorb only 30% of the artificial light distributed in hydroponic greenhouses, nano phosphorous-based light source is developed to cater to the exact wavelength of light required by the plant. It ultimately provides huge savings on energy costs during large-scale production (Witanachichi et al. 2012). Several studies have shown positive effects of exposure of nanoparticles to seedlings in hydroponic culture

(Wang et al. 2021). Enhancement of seed germination, seedling growth, biomass, and vigor in rice is proven to be achieved through seed priming with nanoparticle ZnO (10 mg/L) (Itroutwar et al. 2020), a similar report showed the exposure of nCuO to the hydroponic culture of rice enhanced seedling growth at optimum concentrations (5, 10, 20, and 50 µM) (Tiwari et al. 2019). Additionally, single-walled carbon nanotubes (SWNT) are employed for real-time nanosensing in hydroponically grown *Brassica rapa* and *Oryza sativa* for the detection of 1-naphthalene acetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) uptake (Ang et al. 2021).

Nanotechnology also finds excellent application in plant genetic transformation mechanisms. High transformation efficiency has been reported using nanomaterial-mediated gene delivery without any external physical or chemical means. This method provides advantages over traditional plant transformation systems such as ease of operation, reduced cytotoxicity, and protection of genetic cargos against cellular enzymatic digestion such as nucleases. Nanotechnology-based methods for carrying out gene and chemical delivery are being used in both mammalian and plant systems, however, the success rate in mammalian cells is higher than that of plant systems due to the presence of cell walls that act as biological barriers. Negatively charged cell walls facilitate the binding of cationic nanoparticles more efficiently thus making them a preferred choice for carrying out gene delivery in plants (Ahmar et al. 2021). Many successful plant transformation studies using nanoparticle-mediated transformation methods have been reported such as Gold NPs in rice (Wu et al. 2011), onion (Martin-Ortigosa et al. 2012), and maize (Martin-Ortigosa et al. 2014), and carbon nanotubes in Wheat, cotton, tobacco, arugula, and spinach (Demirer et al. 2019; Kwak et al. 2019).

The rich nutrient profile of leguminous microgreens can further be elevated with the incorporation of these techniques in their cultivation. Nanotechnology has brought together scientists in various fields due to its multidisciplinary applications. It can have multiple applications in microgreen cultivation like enhanced growth of microgreens on exposure to limited doses of nanoparticles, usage of nano biosensors, etc. Furthermore, the ability of genome editing techniques to genetically induce desired traits in several plant systems has the potential to be applied to nonconventional superfoods for their augmentation, such as microgreens. A representative diagram depicting the integration of these techniques in the development of better microgreen variety is given in Fig. 3.

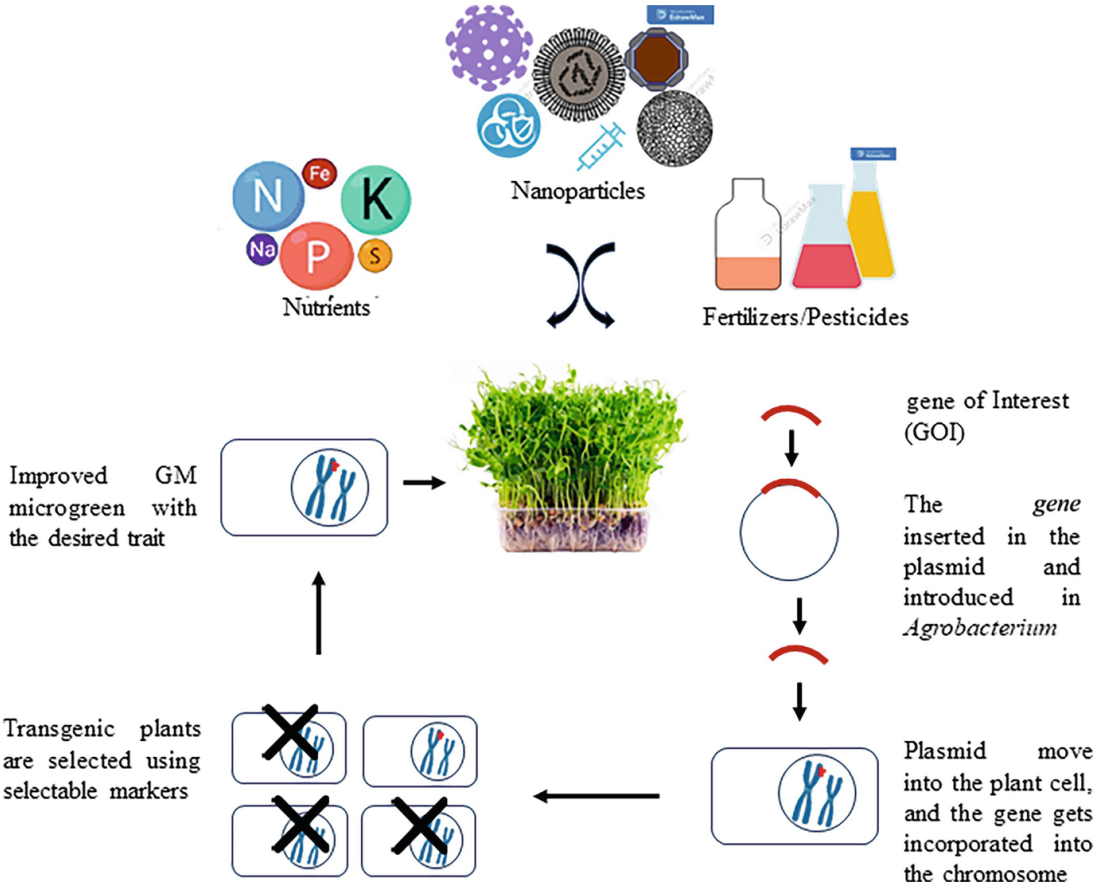


Fig. 3 This diagram depicts the synergistic incorporation of techniques like nanotechnology and genetic modification for the production of elite varieties of microgreens. Nutrient-coated nanoparticles, nano fertilizers, and nano pesticides enable controlled release in plant tissues. Stable transgenic lines of microgreens can be generated by direct incorporation of the desired trait gene

Despite the wide applications that these techniques provide, some challenges need to be addressed. Hydroponic culture and nanoparticle technique require high precision and expertise for handling. As the potential usage of nanoparticles in hydroponics and genetic engineering is increasing, the risk of inducing nano-phytotoxicity also increases. Nanophytotoxicity refers to the toxic impact of the accumulation of nanoparticles on plants. Plants make bridges for the passage of nanoparticles to the environment which can further result in their incorporation into the terrestrial or aquatic food chain. Proper detection methods are needed to give utmost importance while working with NPs (Rastogi et al. 2017). In NP-mediated gene delivery, nanoparticles behave differently with different plant cells and with different biomolecules because their binding affinity is altered by chemical composition, structure, and charge on them. Therefore, their application needs to be optimized for an ideal bioconjugation complex (Ahmar et al. 2021).

5 Impact on Agriculture and Society

Microgreens are a great food option for the modern world due to their high nutritional and phytochemical value as compared to their mature counterparts. Nanotechnology, hydroponics, and genetic engineering together can make a huge difference in the field of microgreens and make it a feasible food option for feeding the growing population. Vertical farming of hydroponically grown microgreens yields high-quality crops with minimal requirement of nutrients and chemicals such as pesticides and fertilizers, and occupies less space as compared to traditional farming techniques thereby, reducing its carbon footprint (Vatistas et al. 2022). It efficiently uses horizontal and vertical space to yield maximum output under controlled environmental conditions. Greenhouse gas emission is a major problem of plant-based food product processing and packaging. It has also been proved that gas released by hydroponic crops is equivalent to 0.11 kg CO2 whereas, in the case of soil-based plants, it is 0.23 kg CO2 (Martinez-Mate et al. 2018). As it is feasible to operate vertical farming through hydroponics or aeroponics in places where soil-based cultivation is difficult, food processing and packaging are least required thereby contributing to the reduction of greenhouse gas emissions. (Parkes et al. 2023).

During recent years there has been a huge change in the trends of agriculture due to innovation and the addition of new cropping technologies to combat the increasing population demands, food diversification, biotic and abiotic constraints, biofortification, urbanization, and climate change issues. It is a major issue in fulfilling the urban population’s food demand in their city, so here microgreen technology along with hydroponics and genetic engineering might give hope to the future population. These technologies also reduces the packaging and transportation problems of food in urban areas. That is why the statistical data of microgreen production and adaptation is trending in an upward direction, globally. However, its market is dependent on consumer acceptance along with the input and output costs. Global market size value is expected to increase from US$11.953 in 2020 to US$19.073 in 2027. The microgreen market is divided into several groups like type of vegetable, growth medium, way of use, distributing units, and geographic distribution.

Short cultivation time along with the low requirement of water, soil, and other inputs make microgreens an ideal business opportunity. Fresh vegetables can be made available in any corner of the world irrespective of geographic areas which are affected by climate change, drought, soil degradation, etc. Due to their high phytochemical and antioxidant properties, they find an important role in the health and cosmetic sector. High nutrients, great flavor, and bright colors allow them to become an important part of restaurants as well. Also the market can be expanded in the coming years with the adoption of vertical farming methods and trained farmers.

In 2017, microgreen farming came up as the most profitable crop (40%) among all other indoor-grown crops. Indoor, hydroponic, and Vertical farming may help farmers to grow microgreens at a large scale in less time and space. The highest market of microgreen production in 2020 was reported in the USA, Asia, Australia, and Canada due to high demand from the cosmetic and food industry and it is expanding globally with time. As stated by the knowledge sourcing intelligence platform, a Sweden-based vertical farming company, Urban Oasis raised 2.2 million US$ to build a fully automated farming system which will rush production and profit by 15–20 times. This mega farm might come as a great competitor for other microgreen-producing farms (Paraschivu et al. 2021).

Major challenges related to traditional agriculture are food security, climate security, and nutrition security. Hydroponics, genetic engineering, and microgreens together can overcome the problems faced during traditional agricultural practices. Microgreens have immense potential to enhance nutritional supplements of the human diet due to the presence of high nutritional compounds. Leguminous microgreens are nutritionally more rich than leguminous sprouts. A study by Irena and team was done on seeds of several legumes (daikon radish, alfalfa, lentil, and fenugreek) to determine the content of polyamines agmatine, spermidine, putrescine, spermine, and cadaverine in seeds, sprouts, and microgreens (Renna and Paradisco 2020). Leguminous microgreens grown hydroponically along with the help of nanotechnology and genetic engineering technology can lead to a drastic increase in the nutritional value and shelf life of microgreens.

While altering the traditional methods of farming, ethical values of the society are also taken into consideration. Ethical concerns are basically the consequences of the applicability of new technology on society and the environment. Genetic engineering is more acceptable in medicine than in the food industry due to ethical reasons. Despite huge advancements in the field of genetic engineering, the development of genetically engineered organisms remains unacceptable in the society (Glass and Fanzo 2017).

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6 Prospects and Conclusion

Microgreens have been widely studied for their potential benefits in the human diet. Leguminous microgreens, with a richness of phytochemical profile, high antioxidant activity, and amino acid content, become even more important for incorporation into daily diet. Considering their low shelf life and scope of generating even higher nutritional value crops, the need of the hour is to explore methods and techniques that work toward the enhancement of nutritional quality and shelf life of microgreens. Hydroponics solves problems related to land unavailability and reduces the energy of packaging and transportation to some extent. It also allows us to

control the quantity and quality of nutrient solutions provided according to the need and type of microgreens. Applications of genetic engineering include the incorporation of desired traits such as resistance to biotic and abiotic factors and enhanced nutrient content. Biofortification of microgreens at the genetic level can be achieved via genome editing technology and nanotechnology. Overall, this study shows the role of hydroponics, nanotechnology, and genetic engineering in the advancement of microgreen agriculture. A combination of all these technologies might revolutionize the field of modern agriculture by increasing the nutritional value of microgreens along with their health benefits. More research in this direction might prove as a modern approach to solving modern problems of food security and sustainability.

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Nanotechnology Innovations in Soilless Microgreen Farming for Sustainable Agriculture

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Abbreviations

NPs Nanoparticles

CNPs Chitosan nanoparticles

FFT Fast Fourier Transform

SeNPs Selenium nanoparticles

LNTs Lignin nanoparticles

ZnONPs Zinc oxide nanoparticles

γ-Fe2O3 NPs Iron oxide nanoparticles

TiO2 NPs Titanium dioxide nanoparticles

CP Cold plasma

FRET Fluorescence resonance energy transfer

PVC Polyvinyl chloride

1 Introduction

Sustainable agriculture has emerged as a critical paradigm in addressing the increasing challenges of global food security, environmental degradation, and resource scarcity. It emphasizes practices that meet the present needs of society without compromising the ability of future generations to meet their own needs. Within this framework, soilless microgreen farming plays a significant role in contributing to sustainable food production (Paradiso et al. 2018; Choe et al. 2018). Soilless microgreen farming aligns with the principles of sustainable agriculture and offers unique contributions to meeting global food demands. Soilless microgreen systems,

such as hydroponics and aquaponics, optimize the use of water and nutrients, providing higher resource efficiency compared to traditional soil-based methods (Christofi et al. 2023). This is particularly crucial in regions facing water scarcity. Soilless microgreen farming allows for year-round production in controlled environments, overcoming seasonal limitations. This consistent supply helps meet the demand for fresh, nutritious greens regardless of external climatic conditions (Christofi et al. 2023). By using controlled inputs and minimal runoff, soilless microgreen farming significantly drops the environmental impact compared to traditional agricultural methods. It minimizes soil erosion, nutrient leaching, and pesticide contamination. Microgreens, which are young and tender seedlings of edible plants, offer a wide range of flavors, textures, and nutrients (Paradiso et al. 2018). Soilless microgreen farming allows for the cultivation of diverse crops, promoting dietary diversity and nutrition. Soilless microgreen farming enables rapid crop turnover, resulting in faster harvest cycles compared to traditional agriculture. This quick turnaround enhances overall productivity and responsiveness to market demands.

Advanced technologies, such as sensors and automated control systems, enable precision agriculture in soilless microgreen farming. This optimizes resource use, minimizes waste, and enhances overall efficiency (Maluin et al. 2021). In addition to traditional farming methods, nanotechnology offers a multitude of opportunities to improve efficiency and address challenges in soilless microgreen cultivation (Abdel-Aziz and Heikal 2021; Maluin et al. 2021). Nanoparticles can enhance nutrient delivery, enable precise environmental monitoring, and improve pest and disease management (Worrall et al. 2018). Nanotechnology holds significant promise in revolutionizing soilless cultivation by enhancing sustainability, productivity, and resilience in various

aspects of the process. Nanomaterials can encapsulate nutrients, protecting them from leaching and enhancing their slow release (Ahmad et al. 2011; Avellan et al. 2019). Controlled release mechanisms, targeted delivery, and improved adhesion to plant surfaces contribute to more effective pest and disease management in soilless cultivation (Maluin et al. 2021). This allows for more precise control over nutrient availability, promoting optimal uptake by plants in soilless systems. Nanoparticles, such as nanoclays or nanoscale organic matter, can be added to soilless growing media to enhance their physical and chemical properties (Ibrahim et al. 2020). Nanoparticles can be engineered to deliver plant growth-regulating substances in a controlled manner. This allows for precise modulation of plant growth and development in soilless systems. Nanotechnology enables the creation of hydrogels with water-absorbing properties. These hydrogels can be incorporated into soilless growth media to improve water retention and reduce the frequency of irrigation, contributing to water efficiency (Ahmad et al. 2011). Nanoscale sensors can monitor soilless environments in real-time, providing data on moisture levels, nutrient concentrations, and other critical parameters. Nanoscale biosensors can detect the presence of pathogens or pests at an early stage, enabling proactive measures to prevent outbreaks (Abdel-Aziz and Heikal 2021).

This review explores the integration of nanotechnology into soilless microgreen farming, focusing on its applications in nutrient management, monitoring, pest and disease management, seed priming, and shelf-life extension. This review explores recent advancements and future prospects to shed light on how nanotechnology can enhance sustainable agriculture and food security through soilless microgreen farming.

2 Heath Benefits of Microgreens

Microgreens are small crops with a height of 10–13 inches and have 7–14-day germination period which offer significant advantages in terms of nutritive value and resource efficiency. Cultivating microgreens, such as broccoli, reduces harvesting time from 100–150 days to just 9 days, cutting water consumption by 150–236 times compared to fully grown counterparts. (Biesalski et al. 2009). They are rich in elements like iron, potassium, calcium, zinc, and bioactive compounds such as antioxidants, ascorbic acid, and anthocyanins (Choe et al. 2018; Paradiso et al. 2018). Mustard, fennel, and radish microgreens exhibit the highest beta carotene concentration, while their total phenolic content is 10 times greater, ranging between 10.71–11.88 mg/g (Weber 2017; Ghoora et al. 2020).

Microgreens also exhibit health benefits indicating their potential to reduce diabetes and cardiovascular diseases (Huang et al. 2016). Fenugreek microgreen extract (2 mg/ml) demonstrated a 70% inhibition of alpha-amylase activity and a 44% increase in glucose uptake (Wadhawan et al. 2018). Brassicaceae family microgreens (kale, mustard, radish, broccoli) showed anti-proliferative effects against colon cancer cells, cultivated through hydroponic farming (Soundararajan and Kim 2018; Fuente et al. 2020).

3 Nanomaterials in Soilless Microgreen Farming

The primary goal of incorporating nanoparticles in soilless farming is to enhance nutrient management, minimize losses, and boost yields. Nanotechnology plays a pivotal role in addressing challenges related to nutrient solutions, aiming to maximize nutrient uptake and enhance crop

productivity and quality. Nanoparticles, with their high specific surface and reactivity (particle size <100 nm), offer plants more soluble and usable nutrients, reducing precipitation and insolubilization common in conventional fertilizers (e.g., phosphate fertilizers). Their unique properties such as physicochemical characteristics and the ability to traverse plant cell walls, facilitate increased nutrient uptake by plant roots. This makes nanoparticles more efficient nutrient carriers than traditional fertilizers, showcasing their promise as a valuable tool, especially in soilless growing systems. In a hydroponic system, chitosan nanoparticles (CNPs) present an environmentally friendly alternative to conventional nutrient solutions. In a particular study, Maluin and colleagues assessed the quality, yield, and nutritional content of hydroponically cultivated Bok Choy (*Brassica rapa* subsp. Chinensis (L.) Hanelt), comparing microgreens to adult vegetables under identical conditions. The research explored the impact of CNP treatments and/or fertilizer supplementation on growth (Maluin et al. 2021; Song et al. 2012).

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4 Nanosensors for Monitoring and Control

Nanosensors present significant potential for optimizing nutrient delivery, water management, and overall system control in agricultural settings, particularly in hydroponic systems (Abdel-Aziz and Heikal 2021). These sensors offer real-time, high-resolution data on nutrient levels within the hydroponic solution, enabling growers to accurately monitor and adjust nutrient concentrations as needed. Integration of nanosensors with smart irrigation systems facilitates direct delivery of nutrients to the plant root zone, ensuring efficient uptake and minimizing waste (Abdel-Aziz and Heikal 2021). Moreover, nanosensors can establish a

feedback loop to continuously monitor and automatically adjust nutrient levels based on plant requirements, thus optimizing nutrient delivery for maximum growth and yield (Christofi et al. 2023).

In addition to nutrient management, nanosensors play an important role in precise irrigation scheduling by accurately measuring soil or substrate moisture levels. This capability helps prevent both overwatering and underwatering, contributing to better water management practices (Abdel-Aziz and Heikal 2021; Christofi et al. 2023). Furthermore, nanosensors monitor environmental parameters such as temperature, humidity, and CO_2 levels, enabling growers to maintain ideal growing conditions for plants. They also detect early indicators of pests or diseases, enabling timely intervention to prevent outbreaks and minimize crop damage (Christofi et al. 2023).

In the context of soilless legume farming and microgreen cultivation, nanosensors offer immense potential for enhancing monitoring and growth optimization. By providing real-time data on nutrient levels, moisture content, pH, and environmental factors, nanosensors enable growers to make informed decisions to maximize crop yield while minimizing resource wastage (Paradiso et al. 2018). Immersible photonic sensors have shown results in evaluating nutrient content in hydroponically cultivated plants like basil microgreens, offering rapid, cost-effective, and on-site assessment capabilities. Immersible photonic sensors determine the nutrient content of microgreens through spectral scanning of the filtered extracts obtained from microgreen homogenates or pulp. The signal analysis is simplified by using Fast Fourier Transform (FFT) algorithms to record the phase change over time occurring due to biomolecular interactions. Various biomolecules present in the microgreen extract can interact with the surface of these

silicon-based nanosensors, thereby translating in a phase change in the recorded spectra and may therefore be indirectly correlated to its nutrient content. Figure 1 depicts nanotechnology-enabled nutrient sensing in microgreens and estimation of time of harvest.

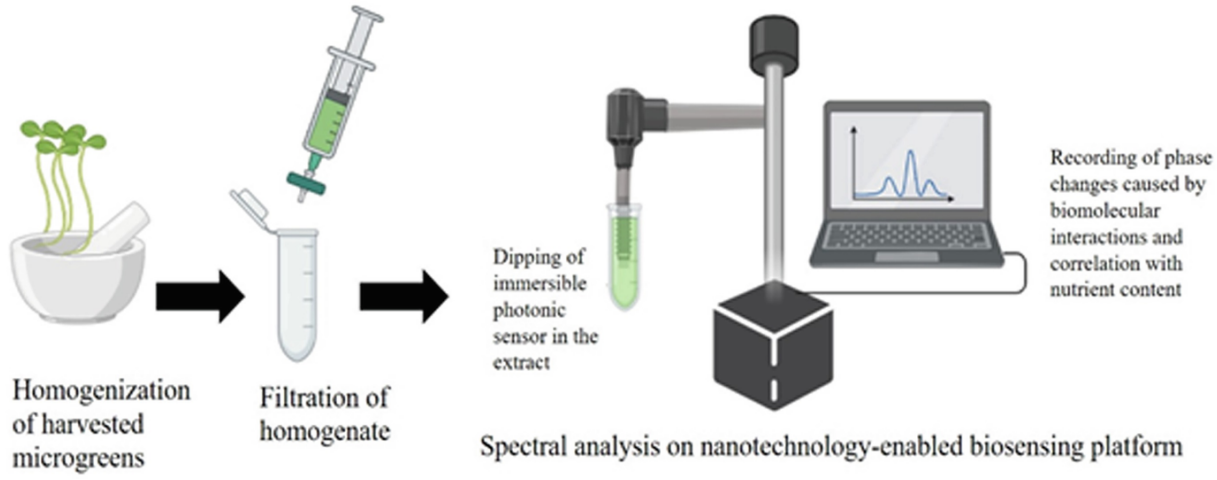


Fig. 1 Nanotechnology-enabled nutrient sensing in microgreens and estimation of time of harvest

Using immersible photonic sensors to evaluate nutrient content in hydroponically cultivated plants, particularly basil microgreens, has shown promising outcomes (Christofi et al. 2023). Additionally, nanosensors are non-invasive and compact, which make them ideal for densely planted trays and ensuring minimal disruption to plant growth (Brambilla et al. 2021). They also serve as real-time detection tools for contaminants, pathogens, and storage conditions in harvested microgreens, enhancing food safety and quality control efforts. Overall, the integration of nanosensors represents a cutting-edge approach to precision agriculture, revolutionizing the way crops are grown and managed for improved sustainability and efficiency (Christofi et al. 2023).

5 Nanoparticles for Nutrient Delivery

Biofortification, especially the use of nanoparticles has been extensively studied to enhance the essential micronutrients like zinc and iron in food crops, including broccoli microgreens (Guardiola-Márquez et al. 2023). Guardiola-Márquez et al. (2023) reported the synergistic interactions between ZnO and ɣ-Fe_2O_3 nanofertilizers combined with biofertilizers in promoting plant growth and enhancing the biofortification of micronutrients and glucosinolates in broccoli microgreens. Additional research has indicated the beneficial effects of zinc (Awan et al. 2021) and iron (Farhan et al. 2023) nanoparticles, along with the bacterial species found in the consortia (Guardiola-Márquez et al. 2023), on the growth characteristics of broccoli. (Awan et al. 2021) observed that the utilization of ZnO NPs resulted in enhanced seed germination (37.5%), root length (56.6%), shoot length (16.6%), and weight (41%) of broccoli seedlings.

Nanoparticles may undergo biochemical transformations in the soil-root interface, affecting their availability to plants. Nanoparticles can be absorbed and translocated within plant tissues, impacting their elemental speciation and accumulation. The enhancement of essential micronutrients through the use of nanoparticles has been widely studied in various food crops, including rice, wheat, maize, broccoli, and chickpeas (Du et al. 2019; Awan et al. 2021; Yang et al. 2021; Farhan et al. 2023). Research conducted by (Sundaria et al. 2019) demonstrated the efficacy of iron oxide nanoparticles in enhancing grain iron content in wheat crops through seed priming, resulting in an increase between 26.8 to 45.7%. Similarly, (Yang et al. 2021) reported improved zinc concentrations in brown rice (13.5–39.4%) through the application of ZnO NPs compared to conventional fertilization methods.

During the germination process of pea seeds, the use of selenium nanoparticles (SeNPs) and Lignin nanoparticles

(LNTs) has been reported to restore the amino acid levels. When used in combination, they effectively restored the amino acid content to a level comparable to that of the control group (Lin et al. 2023). Selim et al. (2022) illustrated that combining SeNPs with β-aminobutyric acid increased the amino acid content in *Medicago interexta* sprouts. Amino acids are vital nitrogen-containing compounds directly absorbable by the human body. Hence, the synergistic use of SeNPs and LNTs could mitigate the decline in amino acid content induced by acetamiprid, presenting a potential approach to preserving the nutritional value of pea sprout production.

Metal-based nanoparticles like ZnO and γ-Fe_2O_3 NPs undergo various surface interactions and biochemical transformations in the soil-root interface, influenced by factors such as capping agents, size, and charge (Lv et al. 2019). These transformations may involve the release of zinc or iron ions from the nanoparticles into the soil, mediated by factors including soil pH, redox conditions, and root exudates. The rhizosphere, enriched with microorganisms and root exudates, plays an important role in promoting the biotransformation of nanoparticles before their absorption (Lv et al. 2019). The utilization of titanium dioxide nanoparticles reported to facilitate cycling and linear phosphorylation processes, thereby enhancing photosynthetic activity of barley. Application of TiO_2 NPs via leaf spraying has been shown to notably enhance the dry matter yield of barley, predominantly by augmenting photoreduction activity (Thabet et al. 2021).

Furthermore, nanoparticles can undergo transformations inside plant tissues, leading to various elemental speciations in plants (Avellan et al. 2019). Absorption and translocation of soil-applied nanoparticles typically initiate at root surfaces and damaged sites, penetrating through apoplastic and symplastic pathways to

reach the vascular system (Cornu et al. 2020). Subsequently, they are transported to the shoot via xylem vessels, with ease of transport influenced by nanoparticle charge (Raliya et al. 2018). Accumulation primarily occurs in aerial plant organs, where nanoparticles can be transformed into ionic forms (Banerjee et al. 2019; Hu et al. 2022).

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6 Seed Priming Approaches in Microgreen

Seed priming is an innovative technique used to improve seed performance and crop yield by treating seeds with specific substances or treatments before planting. This process aims to stimulate early seed germination, improve seedling vigor, and enhance stress tolerance in crops (Do Espirito Santo Pereira et al. 2021; Tondey et al. 2021). One of the emerging methods of seed priming involves the use of nanoparticles, such as iron oxide nanoparticles, to fortify seeds with essential nutrients or to confer specific physiological benefits. Seed nano-priming represents an emerging technology utilizing nanomaterials, primarily nanoparticles, for seed treatment (Kasote et al. 2019; Mahakham et al. 2017). A variety of nanomaterials, such as metallic, biogenic metallic, and polymeric nanoparticles, have shown potential for seed nano-priming (Mahakham et al. 2017; Siddaiah et al. 2018). Nanomaterial-based seed priming stimulates plant growth and improves morphological and metabolic characteristics, facilitating fast root and shoot development. In contrast to traditional seed priming approaches that usually use water or nutrient, hormone, or biopolymer solutions, seed nano-priming employs suspensions or nanoformulations containing nanoparticles (Acharya et al. 2019). These nanoparticles primarily form a coating on the seed surface

(Duran et al. 2017; Montanha et al. 2020). Fungicides or bactericides are used along with the seed coating to defend against pathogens during storage or in the field (Gross et al. 2020). Figure 2 depicts the overall significance of nanoseed priming and its impact on different physiological process.

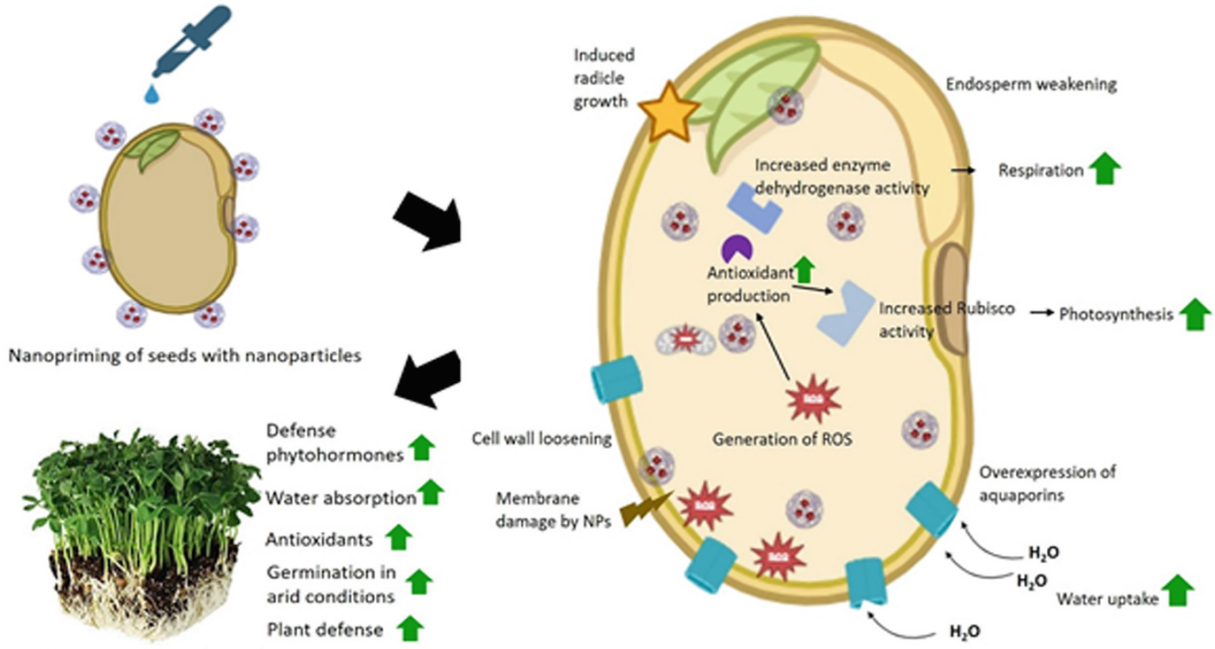


Fig. 2 Overall significance of seed nanopriming and its impact on physiological processes

In the study by Sundaria et al. (2019), the effects of iron oxide nanoparticles on wheat genotypes were investigated. They observed that treating seeds with iron oxide nanoparticles led to increased germination percentage, shoot length, and overall growth parameters in wheat. Although traditional seed priming techniques were not employed, the authors showed that carbon nanotubes could be absorbed by tomato seeds. This resulted in enhanced water uptake and subsequently led to tomato plants with a two-fold increase in flower production (Khodakovskaya et al. 2013). Similarly, other studies have revealed that carbon nanotubes can influence seed metabolism in various plants, including tomato, maize, barley, and soybean by

upregulating the expression of different types of water channel proteins (Villagarcia et al. 2012).

Furthermore, there was a significant accumulation of iron in the grains of treated wheat genotypes, indicating the potential of nanoparticles to enhance nutrient uptake and fortify seeds with essential elements. Another approach to seed priming involves the use of cold plasma (CP) treatment. CP treatment is a pollution-free method that has been shown to improve seed germination, water use efficiency, nutrient uptake, and overall plant yield. CP treatment can positively influence various physiological, biological, and developmental processes in plants, including seed performance, bacterial load on seeds, and alteration of seed coat structures. This treatment enhances seedling growth and stress tolerance, leading to improved crop performance (Sundaria et al. 2019). Additionally, there is a growing interest in integrating machine learning techniques with seed priming approaches. Machine learning algorithms can analyze large datasets to identify optimal seed priming conditions, predict seed performance, and optimize seed treatments for specific crop varieties and environmental conditions. This integration of technology with seed priming offers a sustainable and efficient approach to enhance crop productivity and address global food security challenges. The use of nanoparticles, cold plasma treatment, and machine learning techniques in the procedure of seed priming holds great potential for sustainable agriculture and addressing the increasing demand for food production (Guardiola-Márquez et al. 2023). Additionally, seed nano-priming induces changes in gene expression related to metabolic processes, such as phytohormone production, and enhances the defense system of plants by increasing antioxidant levels and enzyme activities (Duran et al. 2017; Kasote et al. 2019; Li et al. 2019; Acharya et al. 2019). This increased resilience

makes plants more resistant to pests and various environmental stresses when grown under field conditions.

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7 Nanomaterials for Pest and Disease Management

When applied directly to plant seeds, leaves, or roots, nanoparticles have a great deal of potential to fight pathogens and pests like bacteria, fungi, viruses, and insects. Prominent metal nanoparticles with antibacterial, antifungal, and antiviral activities include copper, zinc oxide, silver, and titanium dioxide (Gogos et al. 2012; Kah and Hofmann 2014; Kim et al. 2018). Silver nanoparticles have become more and more popular due to recent advances, especially in “green synthesis,” which has demonstrated considerable antifungal action against a variety of diseases and even total inhibition of specific viruses on plant leaves (Elbeshehy et al. 2015; Rafique et al. 2017). However, issues with toxicity, soil interaction, and production techniques prevent them from being widely used (Kah and Hofmann 2014; Mishra and Singh 2015). Other frequently used metal nanoparticles include gold, copper, and titanium dioxide; however, these are mostly used in fertilizer applications, with little research being done on their ability to manage plant diseases (Kah and Hofmann 2014; Sadeghi et al. 2017). Prominent research has demonstrated the antibacterial capabilities of copper, silver, and titanium dioxide nanoparticles in addition to the effectiveness of aluminium nanoparticles as an insecticidal dust (Sadeghi et al. 2017; Worrall et al. 2018). Plant tissues develop viral resistance in response to chitosan nanoparticles, which are known for their advantageous biological characteristics and low toxicity (Cota-Arriola et al. 2013). This resistance provides protection against a variety of viruses. Though less efficient against bacteria,

they also have antibacterial qualities against a variety of fungal infections (Malerba and Cerana 2016). Chitosan's antibacterial properties are a result of mechanisms such as agglutination and membrane disruption (Malerba and Cerana 2016). Furthermore, the biopolymer like chitosan works well against a variety of pests, including psyllids, nematodes, leafworms, and aphids (Malerba and Cerana 2016). Chitosan has great potential as a stand-alone nanoparticle and as a possible nanocarrier due to its many advantages. In a different field trial, Song et al. (2012) treated *Brassica chinese* with silica-encapsulated chlorfenapyr for three days in order to obtain comparable or better control against the diamondback moth (*Plutella xylostella*). According to another study, termites that were fed fipronil loaded into nanoparticles with an oil core and a silica shell that was thicker than usual were able to sustain the release of the bait without experiencing an initial burst, potentially enabling the worker termites to return the bait to the nest (Wibowo et al. 2014). The silica-loaded fipronil increased the 100% mortality window by 3 days in comparison to the commercial pesticide, enabling greater colony removal. Jenne et al. (2018) examined the effects of loading azadirachtin onto zinc oxide or chitosan nanoparticles and evaluated the effectiveness over a 180-day period in storage settings for groundnut bruchids. ZnO nanoparticle-loaded Neem seed kernel extract contained groundnut bruchids better than other tested formulations by 54.61% as reflected by loss in weight. The use of nanoparticles or nanocarriers as protective agents against pests and illnesses to protect plants is shown in Fig. 3.

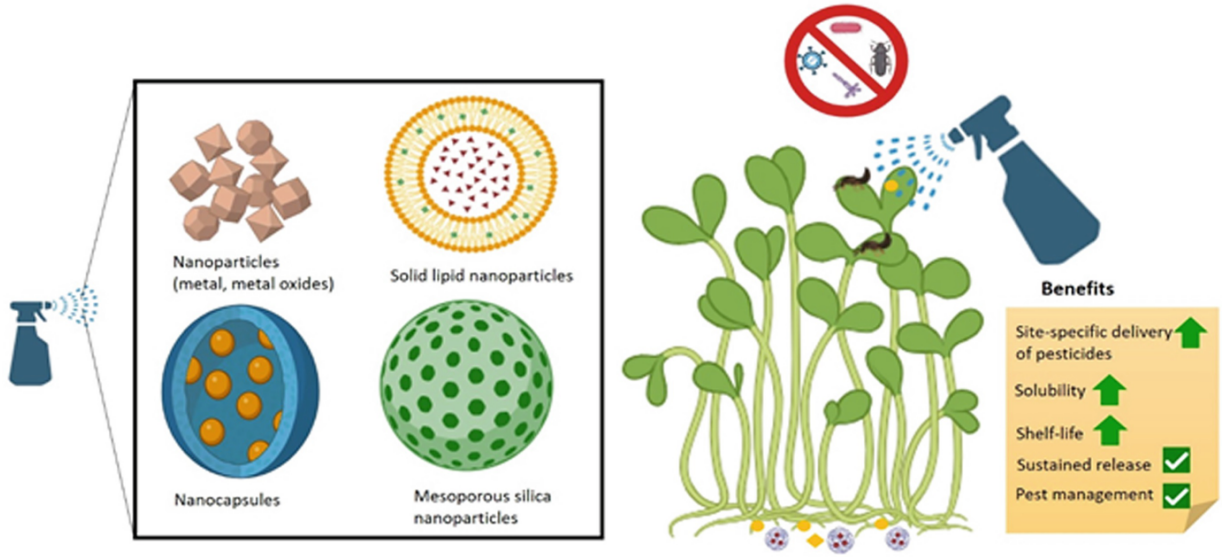


Fig. 3 Application of nanoparticles and nanocarriers of pest control materials for plant protection through foliar spray or root exposure treatment

8 Nanotechnology's Role in Extending Shelf-Life of Microgreens

Microgreens are delicate and highly perishable, making them susceptible to quality deterioration and spoilage. Nano packaging methods offer innovative solutions to address these challenges by enhancing shelf life and preserving quality. By utilizing innovative materials and techniques, nano packaging helps mitigate quality deterioration, microbial growth, and metabolic processes, ensuring the freshness and safety of microgreens for consumers (Sharma et al. 2023). Continued research and development in this field are essential to further optimize nano packaging methods for microgreen preservation. The use of nano packaging, coatings, and bubbles significantly extends the shelf life of harvested microgreens while effectively restricting microbial invasion, physiological maturity, gaseous exchange, and solvent discharge (Idbella et al. 2023). Various nano coatings, including protein SiOx nano composite films, zinc oxide nanoparticle-coated poly

vinyl chloride (PVC) films, protein isolate coatings with zinc oxide, chitosan nano composite films, and many others, have demonstrated efficacy in preserving microgreens' quality and freshness (Chausali et al. 2022). These nano materials create a protective barrier against external factors, preventing deterioration and prolonging storage life. Additionally, the incorporation of essential oils, enzymes, and antimicrobial agents further enhances their preservation capabilities. Overall, nano packaging and coatings offer a promising solution for maintaining the quality and safety of harvested microgreens.

Nano coatings such as protein SiOx nano composite film coating, zinc oxide nanoparticle-coated PVC film, and chitosan nano composite film coating contribute to extended shelf life and prevent microbial invasion in harvested microgreens (Idbella et al. 2023). Various nano coatings, including those containing chitosan, silicon nanoparticles, titanium oxide nanoparticles, and essential oils, demonstrate the ability to extend storage life and prevent quality deterioration in microgreens. Nano packaging methods involving materials like biodegradable poly lactide-co-glycolide nanofiber film with thymol, polyethylene and nano powder-silver, chitosan-titanium oxide nanocomposite film, and silver nanoparticle-polyethylene bags influence physiological maturity, physiochemical quality, and shelf life in harvested microgreens (Chausali et al. 2022; Li et al. 2022) Techniques such as natural modified atmosphere packaging with cellulose-silver nanoparticles, nanocomposite packaging materials containing titanium oxide, silver nanoparticles, and silicon oxide, as well as other innovative packaging approaches, transform storage life, quality, and physiological maturity in harvested microgreens. Nanoparticles of selenium, zinc oxide, calcium carbonate, hydrogel polymer, titanium oxide, magnesia, zinc sulfate, ferrous oxide, ferric oxide, and yttrium III oxide integrated

into polymers like polyethylene terephthalate, polylactic acid, polypropylene, polystyrene, low-density polyethylene, and high-density polyethylene improve the shelf life and antimicrobial activity of microgreens (Sharma et al. 2023).

Nano packaging materials mitigate browning index, total phenolic content, malondialdehyde content, and control titrated chemical compounds or ascorbic acid, leading to improved shelf life and quality in microgreens. Nano packaging materials improve shelf life by reducing high gaseous exchange, high respiration, deterioration, water loss, and gas permeability in harvested microgreens. Specific nanoparticles, such as zinc oxide, calcium carbonate, silicon oxide, and silver nanoparticles, when integrated into packaging materials, demonstrate positive effects on shelf life, quality, and physiological attributes of microgreens (Sharma et al. 2023). The utilization of chitosan-based complex nanoparticles, such as chitosan-hexaconazole nanoparticle, chitosan-dazomet nanoparticle, and chitosan-hexaconazole-dazomet nanoparticle, demonstrates notable contributions to both shelf life extension and antifungal activity in harvested microgreens (Ifijen et al. 2022; Deshmukh et al. 2023). Nano packaging materials play a crucial role in improving the quality and shelf life of harvested microgreens by addressing various factors such as browning, phenolic content, and malondialdehyde accumulation (Sharma et al. 2023). These materials also regulate key chemical compounds like ascorbic acid and mitigate issues related to high gaseous exchange, respiration rates, and water loss (Sharma et al. 2023). For instance, zinc oxide nanoparticle powder integrated with polyvinyl chloride effectively prevents morphological decay, malondialdehyde accumulation, and microbial invasion, thereby enhancing shelf life in microgreens (Srividya et al. 2017; Vannozzi et al. 2020). Similarly, calcium carbonate nanoparticles combined with low-density polyethylene prolong the shelf life of Chinese

yam microgreens by regulating enzyme activity (Zhao et al. 2022). Furthermore, silicon oxide nanoparticle-chitosan complex packaging films improve shelf life in tomato microgreens by reducing total soluble solids, weight loss, and softening (Khalid et al. 2022). Silver nanoparticle and zinc oxide nanoparticle combinations, along with vacuum packaging, modify shelf life in microgreens such as pak choi and radish, especially considering storage temperature (Zhang et al. 2017). Chitosan-based complex nanoparticles, like chitosan-hexaconazole and chitosan-dazomet nanoparticle formulations, exhibit both shelf life extension and antifungal activity in harvested microgreens (Zuverza-Mena et al. 2016). Moreover, nanosensor technologies, employing carbon-based electrochemical, carbon nanotubes, plasmonic, nanowire, fluorescence resonance energy transfer (FRET), and antibody-based sensors, provide real-time detection of contaminants, pesticides, pathogens, and storage conditions, contributing to the quality assurance and safety of harvested microgreens (Maluin et al. 2021). Figure 4 illustrates the utilization of nano packaging films to prolong the shelf life of microgreens.

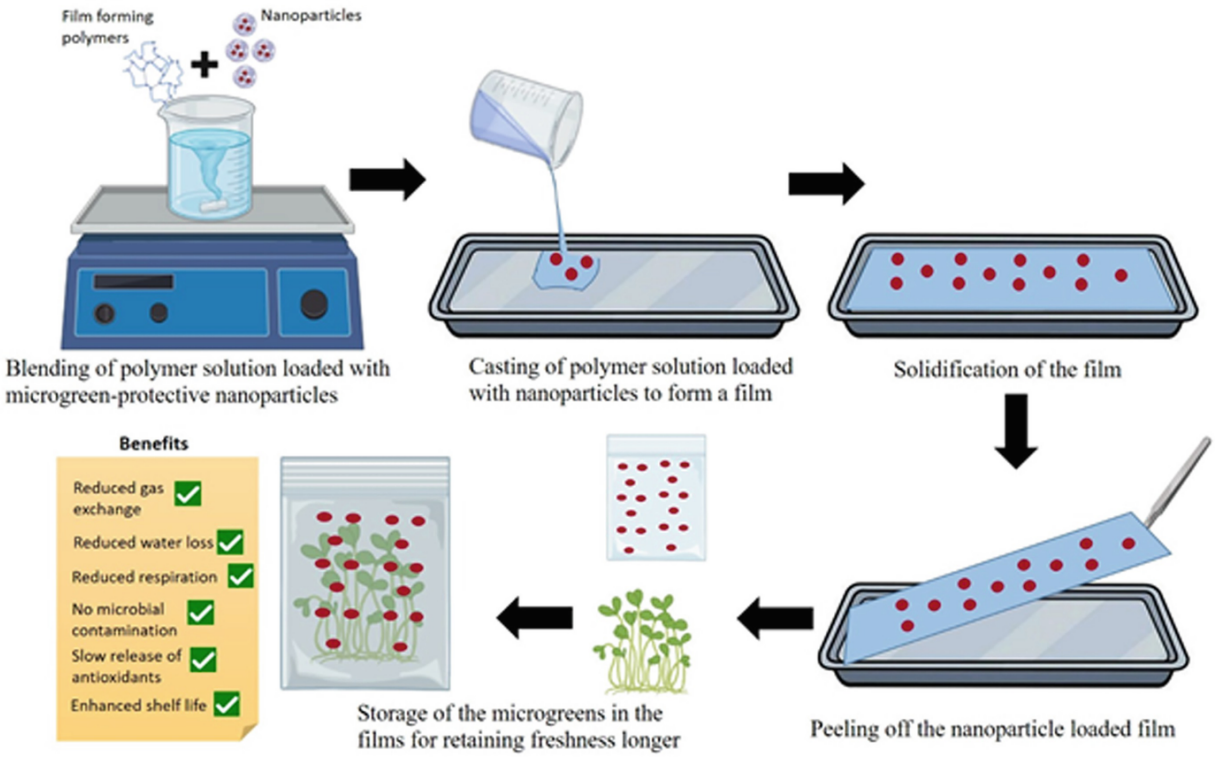


Fig. 4 Nano packaging films applied to extend the shelf life of microgreens

9 Conclusion and Future Directions

The integration of nanotechnology with precision agriculture techniques holds significant promise for the future of microgreen farming. By leveraging nanosensors for real-time monitoring of nutrient levels, environmental conditions, and pest/disease presence, growers can optimize resource usage, maximize yields, and ensure crop health. Continuous research toward the development of advanced nanomaterials for nutrient delivery, pest management, and shelf-life extension will be crucial. Innovations in nanofertilizers, nanopesticides, and nano-packaging materials can further enhance the efficiency, sustainability, and safety of microgreen production. Integration of machine learning algorithms and automation technologies with nanosensor networks can enable autonomous decision-making and precise control over

farming operations. This approach can lead to optimized resource allocation, reduced labor requirements, and increased productivity. Nanotechnology offers a promising means for biofortification, enhancing the nutritional content of microgreens and other crops. Future research can explore the potential of nanoparticles to fortify microgreens with essential micronutrients and bioactive compounds producing functional foods with enhanced health benefits. As nanotechnology continues to advance in agriculture, establishing robust regulatory frameworks and safety standards will be essential to ensure consumer safety and environmental sustainability. Research efforts should focus on evaluating the long-term impacts of nanomaterial use on ecosystems and human health.

In conclusion, the integration of nanotechnology holds immense potential to revolutionize microgreen farming, addressing key challenges related to nutrient management, pest control, shelf-life extension, and overall sustainability. Through the development of advanced nanomaterials, precision farming techniques, and innovative packaging solutions, growers can optimize resource usage, enhance crop quality, and meet the growing demand for nutritious and safe food as depicted in Fig. 5. However, further research, collaboration, and regulatory oversight are necessary to unlock the full benefits of nanotechnology in microgreen production while ensuring environmental stewardship and consumer protection. With continued innovation and strategic implementation, nanotechnology can pave the way for a more efficient, resilient, and sustainable microgreen industry, contributing to global food security and human health.

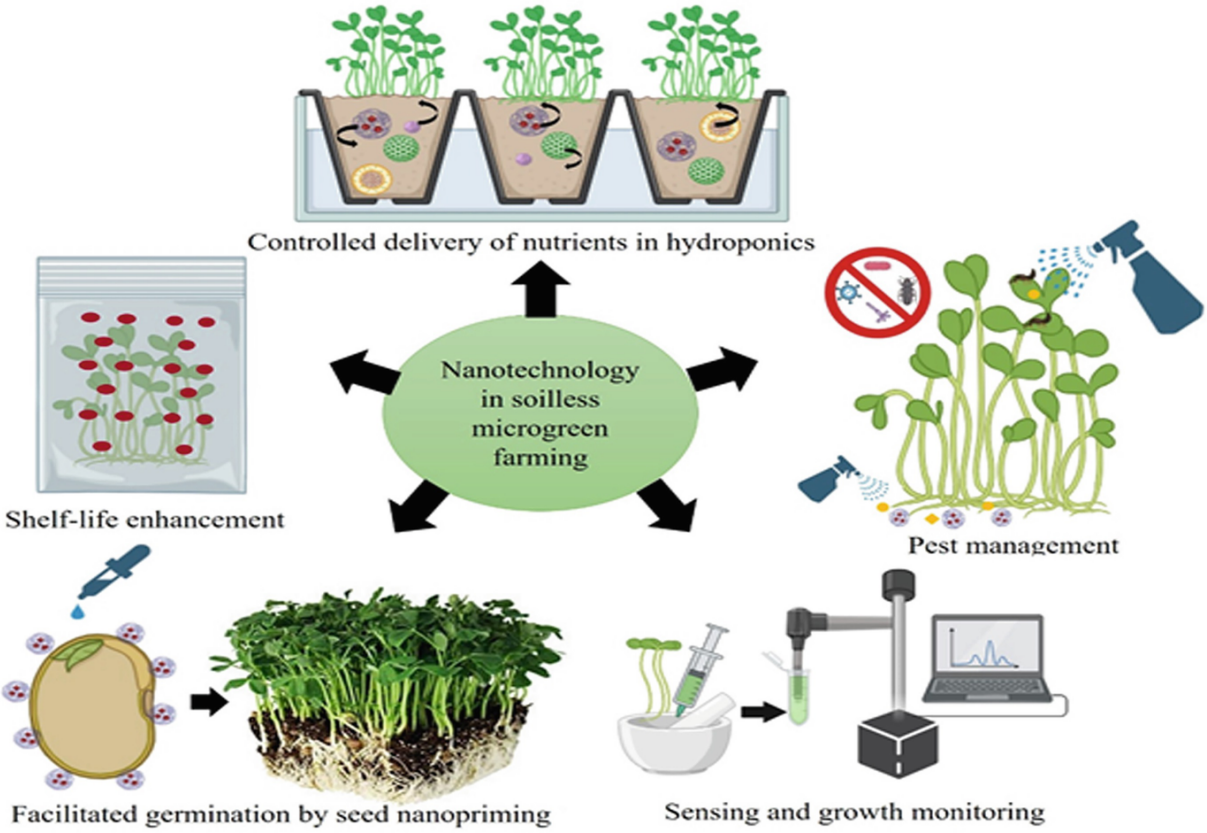


Fig. 5 Innovative applications of nanotechnology in soilless microgreen farming

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Potential Effects of Nanotechnology for the Enhancement of Leguminous Microgreens Quality, Productivity and Sustainability

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Keywords Legume microgreens – Nanotechnology – Quality enhancement – Shelf life

Abbreviations

CNTs Carbon nanotubes

CRISPR-CAS9 Clustered regularly interspaced short palindromic repeats

LDPE Low density polyethylene

MAP Modified atmosphere packaging

PVC Polyvinyl chloride

SiO Silicon oxide

TiO_2 Titanium dioxide

UV Ultraviolet

ZnO Zinc oxide

Sadhika Bose and Khushi Dutta are contributed equally, “joint first author”.

1 Introduction

Microgreens can be defined as young, fragile and immature plants whose phytochemical content is high and whose harvest time varies between 7 and 21 days (Ćira et al. 2018a, b). They are a rich source of phytochemical such as alpha tocopherol, phylloquinone, beta-carotene, ascorbic acid, vitamins and many other elemental minerals (Ćira et al. 2018a, b). This rich nutrient profile positions them as a potential source of vitamins, minerals, and bioactive compounds with medicinal properties (Li et al. 2019a, b). Studies suggest micro-greens may help alleviate disease conditions such as diabetes and also prevent inflammation (Li et al. 2019a, b). Their delicate nature necessitates proper care during cultivation to avoid damage from environmental factors like temperature, humidity, and nutrient availability (Rouphael et al. 2018). Shelf life refers to the storage methods employed to maintain micro-green quality after harvest and minimize spoilage during marketing (Singh et al. 2021a, b). This involves monitoring and managing storage conditions to ensure the product remains fresh and marketable (Singh et al. 2021a, b).

There are various methods of producing microgreens, some of which utilize soilless cultivation techniques (Rouphael et al. 2018). These methods (Fig. 1), along with other factors like storage conditions, shelf life, and preservation methods, significantly impact the final product sold in the market (Singh et al. 2021a, b). Storage duration directly affects micro green quality and marketability. While proper storage helps maintain quality, extended storage can lead to degradation and loss of freshness (Singh et al. 2021a, b). Common issues associated with long-term storage include wilting, chlorosis, and tissue necrosis, all contributing to a decline in micro green quality (Singh et al. 2021a, b). These are all considered abiotic factors affecting micro-greens. For proper micro green packaging, several criteria are essential, including preventing gas exchange and inhibiting enzymatic

processes that can degrade the product (Duncan et al. 2012). Nanoparticles are gaining significant attention in micro green packaging, preservation, and shelf-life extension (Alagaratnam et al. 2019). These nanoparticles can not only manage and optimise plant metabolism, supporting maturation, but also control environmental conditions to maintain optimal quality and freshness (Alagaratnam et al. 2019). Studies have shown that nanoparticle application improves product shelf life, increases quality, and acts as an antimicrobial agent to prevent spoilage caused by microbial contamination (López-Rubio et al. 2020). Scientific literature documents the effectiveness of nanoparticle-mediated packaging (nano-packaging) and nanoparticle coatings on micro green products in promoting shelf-life extension (Alagaratnam et al. 2019). These nanoparticle-based treatments can also inhibit microbial growth and contamination, improve gas diffusion rates, and influence micro green plant maturity (López-Rubio et al. 2020).

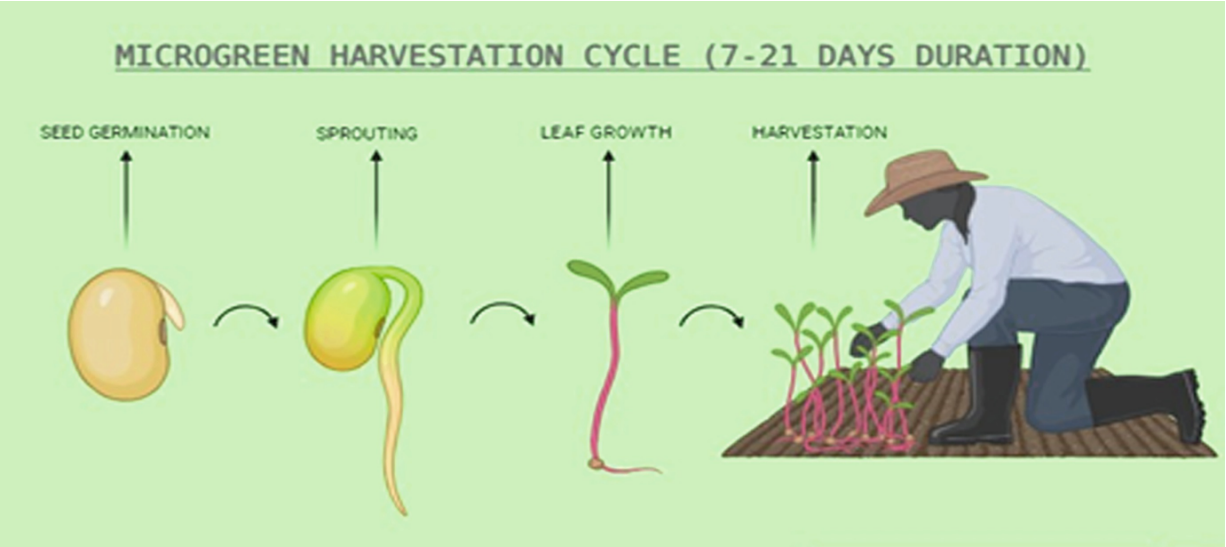


Fig. 1 A pictorial illustration of the harvest cycle (7–21 days) of microgreens

2 Leguminous Microgreens

These are microgreens specifically grown from legumes (plants in the Fabaceae family) (Table 1) (Lewis et al. 2005). Legumes are known for their seeds being rich in protein and fiber (Mudryj et al. 2014). Common examples of legumes include peas, beans, lentils, and peanuts (Table 1) (Lewis et al. 2005). All leguminous microgreens are microgreens, but not all microgreens are

leguminous. There are microgreens grown from other plant families like Brassicaceae (cabbage family—kale, broccoli) (Pascual et al. 2010), Asteraceae (daisy family—sunflowers) (Ling et al. 2018), and Apiaceae (carrot family—cilantro, dill) (Bartolome et al. 2012).

Table 1 A compendium of leguminous microgreens presenting diversity in pigments and curating a taxonomically categorised table elucidating genus, species and colour characteristics

Commercial name	Family	Genus	Species	Plant colour
Pea shoots	Fabaceae	Pisum	sativum	Green
Alfalfa sprouts	Fabaceae	Medicago	sativa	Green
Clover sprouts	Fabaceae	Trifolium	pratense	Green
Lentil sprouts	Fabaceae	Lens	culinaris	Brown
Chickpea sprouts	Fabaceae	Cicer	arietinum	Yellow
Mung bean sprouts	Fabaceae	Vigna	radiata	Green
Black bean sprouts	Fabaceae	Phaseolus	vulgaris	Black
Adzuki bean sprouts	Fabaceae	Vigna	angularis	Brown/red
Green bean sprouts	Fabaceae	Phaseolus	vulgaris	Green
Soybean sprouts	Fabaceae	Glycine	max	Green
Cowpea microgreens	Fabaceae	Vigna	unguiculata	Green
Guar microgreens	Fabaceae	Cyamopsis	tetragonoloba	Green
Lima bean sprouts	Fabaceae	Phaseolus	lunatus	Green/white
Winged bean sprouts	Fabaceae	Psophocarpus	tetragonolobus	Green
Hyacinth bean sprouts	Fabaceae	Lablab	purpureus	Green/purple
Pigeonpea sprouts	Fabaceae	Cajanus	cajan	Green
Yardlong bean sprouts	Fabaceae	Vigna	unguiculata	Green

3 Nutritional Value and Health Benefits

Legume microgreens offer a variety of nutritional benefits and health-promoting properties. These little powerhouses are packed with essential nutrients, including vitamins, minerals, antioxidants, and phytochemicals (Ćira et al. 2018a, b). They are known to provide a concentrated source of nutrients that can support overall health and well-being (Ćira et al. 2018a, b). Studies have shown that legumes contain bioactive compounds such as polyphenols that exhibit antioxidant, anti-inflammatory, anti-cancer,

antimicrobial and hypoglycemic effects (Alegría et al. 2019). Here is some specific health benefits associated with consuming microgreens (Table 2).

Table 2 Research on bioactive compounds in legumes and their effects on human health

Health benefit	Description
Blood regulation	Legumes can help regulate blood lipid levels and blood pressure (Pereira et al. 2010).
Boosting immunity	The high nutrient content of microgreens, which are young legume seedlings, can contribute to a stronger immune system (Ćira et al. 2018a, b).
Gut health	Microgreens can support the health of the gastrointestinal tract due to the presence of beneficial bacteria (Ćira et al. 2018a, b).
Heart health	The antioxidant properties of polyphenols in legumes may help prevent cardiovascular disease (Alegría et al. 2019).

4 Vegetable Versus Legume Microgreen Nutrient Comparison (with Estimated Values)

A Table 3 is given below comparing some common vegetables with their leguminous microgreen counterparts, including estimated nutrient values per 100 g. It's important to note that not all vegetables have direct legume microgreen equivalents, and these are just approximations.

Table 3 A comparative study demonstrating the nutrient content of a specific vegetable and their corresponding leguminous microgreen alternatives highlighting those leguminous microgreens often possess higher levels of certain vitamins and minerals compared to their mature vegetable counterparts

Vegetable	Legume microgreen	Vegetable nutrient	Legume microgreen nutrient
Broccoli	Clover	Vitamin C: 89 mg	Vitamin C: 120 mg Vitamin K: 1000 mcg Iron: 2.7 mg Magnesium: 88 mg
Beet	Mung bean	Vitamin C: 6 mg Folate: 188 mcg Potassium: 422 mg	Vitamin C: 150 mg Vitamin K: 750 mcg Iron: 3.5 mg Protein: 25 g

Vegetable	Legume microgreen	Vegetable nutrient	Legume microgreen nutrient
Radish	Alfalfa	Vitamin C: 6 mg Potassium: 265 mg	Vitamin A: 5000 IU Vitamin C: 180 mg Vitamin K: 800 mcg Iron: 4 mg Manganese: 1.2 mg
Carrot	Pea shoot	Vitamin A: 11000 IU Potassium: 422 mg	Vitamin A: 15000 IU Vitamin C: 200 mg Vitamin K: 1200 mcg Iron: 2.2 mg
Spinach	Lentil	Vitamin K: 838 mcg Vitamin A: 5220 IU Magnesium: 157 mg	Vitamin A: 9000 IU Vitamin C: 180 mg Vitamin K: 1500 mcg Iron: 7.3 mg Protein: 30 g

5 Importance of Shelf Life and Quality Enhancement

The quality and shelf life of legume microgreens are critical factors to ensure consumer satisfaction and market competitiveness (Singh et al. 2021a, b). Pesticide residues on legume seeds can pose dietary risks and negatively affect the growth and nutritional quality of sprouts (Li et al. 2019a, b). The development of strategies to increase the quality and durability of microgreens is necessary to maintain their nutritional value and sensory properties (Xiao et al. 2018a, b).

5.1 Extending Shelf Life: Promising Techniques

Research efforts are actively exploring quality improvement techniques to extend the shelf life of legume microgreens. These techniques aim to maintain a fine balance between nutritional content, flavour profile and visual appeal. Promising approaches include:

5.1.1 Modified Atmosphere Packaging (MAP)

This technique creates a controlled atmosphere (Fig. 2) in the package that reduces respiration rate, ethylene exposure and

microbial growth, thereby increasing shelf life (Singh et al. 2021a, b).

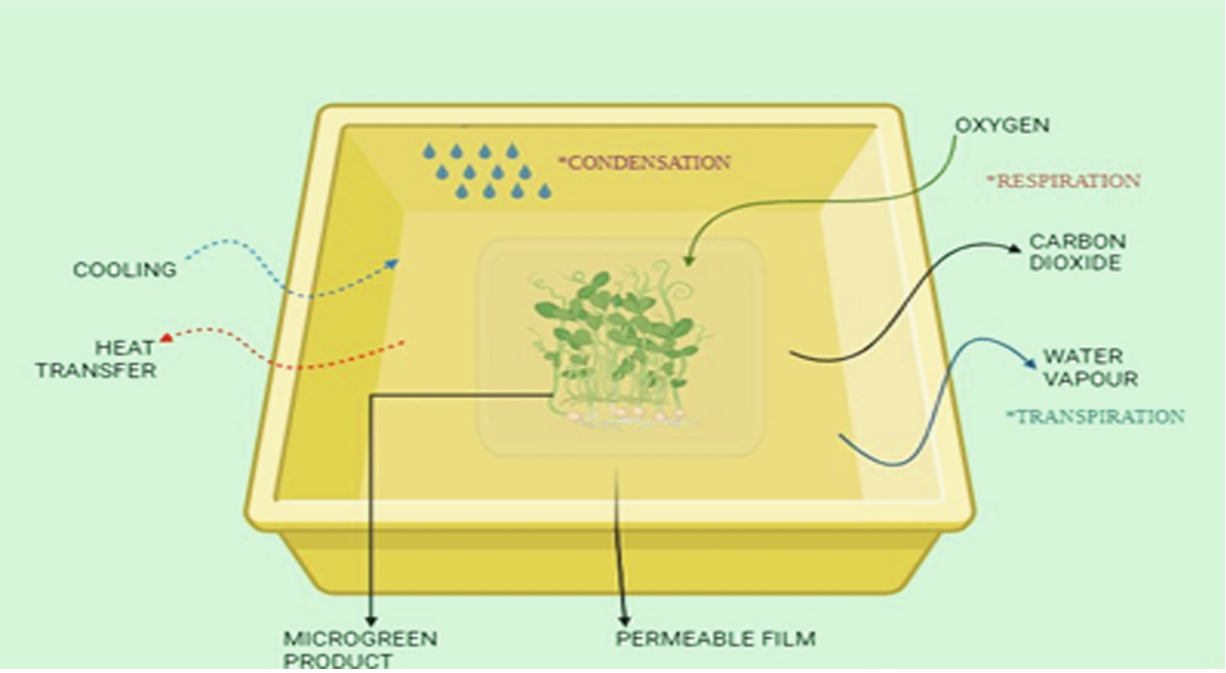


Fig. 2 A pictorial illustration of modified atmospheric packaging (MAP) system

5.1.2 Low-Dose Irradiation

This method can inactivate spoilage microorganisms without compromising the nutritional value of microgreens (Li et al. 2019a, b).

5.1.3 Natural Antimicrobial Coatings

Derived from plants or essential oils, these coatings offer an environmentally friendly option to extend shelf life by inhibiting microbial growth (Wasmund et al. 2019a, b).

5.2 Impact of Storage Conditions on Leguminous Microgreens

Storage conditions play a critical role in determining the quality and shelf life of legume microgreens (Singh et al. 2021a, b). Factors such as temperature, humidity, exposure to light, and air circulation can significantly affect the freshness and nutritional content of microgreens (Singh et al. 2021a, b). Improper storage can lead to moisture loss, wilting, microbial growth, and nutrient degradation (Xiao et al. 2018a, b). It is essential to store

microgreens in optimal conditions, such as refrigeration or controlled atmosphere storage, to maintain quality and extend shelf life (Singh et al. 2021a, b). Proper handling and storage practices can help preserve the texture, flavour, and nutritional value of microgreens (Xiao et al. 2018a, b). Storage atmosphere can affect respiration rate and enzyme activity in microgreens (Jiang et al. 2019a, b). Creating an optimal modified atmosphere (MAP) environment with a specific balance of oxygen and carbon dioxide is crucial for extending shelf life (Singh et al. 2021a, b). For instance, legumes like lentil sprouts contain polyphenol oxidases that can influence enzymatic browning during storage (Singh et al. 2023a, b). Chemical treatments used during storage can affect the sensory properties of legumes and potentially compromise their quality (Wasmund et al. 2019a, b). Refrigerated storage conditions can influence the bioactive compounds and microbial quality of legume microgreens, particularly those from the Brassica family (Martínez-Romero et al. 2018). Washing and dewatering processes before packaging can help extend the shelf life of sprouts and microgreens (Wasmund et al. 2019a, b). Commercialising microgreens directly in containers can ensure higher quality freshness and bypass post-harvest handling processes that might compromise quality (Martínez-Romero et al. 2018).

5.3 Challenges in Maintaining Shelf Life

The primary obstacle lies in the natural biological composition of microgreens. Their high metabolic rate leads to rapid respiration, which quickly depletes their energy reserves and shortens their durability (Singh et al. 2021a, b). This rapid utilization of sugars and starches not only destroys their texture and taste, but also reduces their nutritional value (Singh et al. 2021a, b; Xiao et al. 2018a, b). Factors such as genotype, growing season, cultivation systems, and harvest time can affect the post-harvest quality of microgreens by affecting the accumulation of non-enzymatic antioxidant compounds (Ćira et al. 2018a, b). In addition, legume microgreens have a high level of enzymatic activity that can accelerate processes such as chlorophyll degradation and browning. This not only reduces their visual appeal, but also degrades essential nutrients (Singh et al. 2023a, b). To make things even more complicated, some types of legumes are particularly sensitive to ethylene gas, a natural ripening agent

commonly released by fruits and vegetables. Ethylene exposure triggers rapid senescence (senescence), further reducing the quality of microgreens (Singh et al. 2021a, b). Environmental factors also play a critical role in shelf life. Fluctuations in temperature and humidity can significantly affect the delicate condition of these microgreens. High temperatures accelerate enzyme activity and microbial growth, while low humidity leads to dehydration and wilting (Singh et al. 2021a, b). Perhaps the most worrisome challenge lies in the area of microbial growth. Due to their high moisture content and ideal growing temperature, microgreens are susceptible to contamination by foodborne pathogens, posing a potential health risk. Even in the absence of pathogens, the growth of common bacteria and fungi (mesophilic microflora) can degrade the quality and shorten the shelf life of these microgreens (Wasmund et al. 2019a, b). Despite the challenges mentioned earlier, researchers are actively exploring solutions to improve the shelf life of legume microgreens. Optimizing growing conditions, including light, temperature, and nutrient availability, can influence the physiological state of microgreens and potentially increase their post-harvest resilience (Ćira et al. 2018a, b). In order to minimize the risk of pathogen contamination throughout the production chain, implementing strict hygiene procedures is essential (Wasmund et al. 2019a, b). Additionally, applying natural or synthetic edible coatings can create a physical barrier against moisture loss, microbial infiltration, and ethylene exposure (Singh et al. 2021a, b). Strategies are also being developed to mitigate the impact of stressors like pesticide residues and environmental factors on microgreen plant quality. These might involve using selenium nanoparticles and lentinans to increase shelf life and preserve nutritional value (Singh et al. 2024). Finally, postharvest treatments such as pulsed light-dose irradiation are being investigated for their potential to control microbial growth without compromising the quality of the microgreens (Jiang et al. 2019a, b). By overcoming these challenges and implementing effective shelf-life extension strategies, legume microgreens can reach consumers as safe, fresh, and nutrient-packed powerhouses. This will benefit health-conscious consumers seeking delicious additions to their diet and pave the way for a more sustainable and profitable future for micro green legume producers.

6 Nanotechnology in Agricultural Farming

Nanotechnology, the manipulation of matter at the atomic and molecular scale, is revolutionizing various industries, and agriculture is no exception (Azam et al. 2019). This burgeoning field offers a plethora of innovative tools with enormous potential to address challenges in modern agriculture (Singh et al. 2017). One particularly exciting area of research focuses on applying nanotechnology to enhance the quality and shelf life of legume microgreens (Xiao et al. 2018a, b). Legume microgreens, the young seedlings of lentils, peas, beans and other legumes that have gained recognition for their concentrated nutrition and unique taste (Xiao et al. 2018a, b), face a major hurdle—maintaining freshness and quality throughout the supply chain. Nanotechnology is emerging as a potential game changer. Encapsulated nanoparticles could deliver essential nutrients and enzymes directly to microgreens, optimizing utilization and potentially increasing their nutritional value (Liu et al. 2010). Nano films applied to seeds or microgreens can act as a barrier against moisture loss and ethylene gas, both of which accelerate senescence (Singh et al. 2021a, b). Additionally, smart packaging with built-in sensors could monitor and even regulate storage conditions for optimal shelf life (Wong et al. 2017). Finally, nanotechnology offers a solution to microbial contamination. Antimicrobial nanoparticles in coatings or packaging materials could provide continuous defense, while nano biosensors could enable rapid detection of pathogens for early intervention (López-Ruiz et al. 2018). While further research is essential to ensure safety and address environmental issues (Kuppusamy et al. 2016), the responsible development of nanotechnology holds immense promise for the legume microgreens industry, enabling producers to deliver fresh, high-quality microgreens and support a more sustainable food system (Fig. 3).

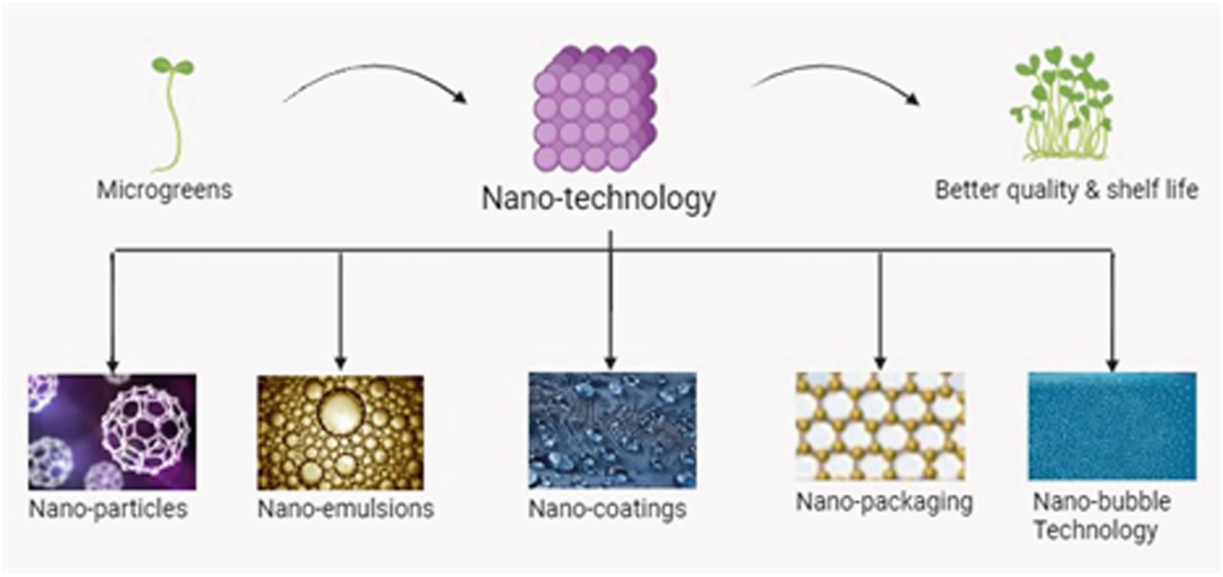


Fig. 3 This figure presents a schematic overview of various nanotechnologies employed across the food production cycle. It highlights applications in farming (nanoparticle nutrient delivery), processing/packaging (nanocoatings for extended shelf life), delivery/storage (nano-emulsions for preservation), and sensing/monitoring (nanosensors for quality control). Arrows depict the interconnectedness of these technologies

6.1 Nanoparticles

These microscopic particles can deliver nutrients directly to plant roots or leaves, increasing nutrient uptake efficiency and minimizing waste. In addition, nanoparticles can be engineered to act as targeted pesticides or fungicides, reducing overall chemical use and environmental impact.

6.1.1 Nanoclays

Nanoclays are nanoparticles derived from natural clay minerals such as montmorillonite. These nanoparticles can improve soil structure, water retention and nutrient availability, promoting healthier root development and overall plant growth in microgreen legumes (Rahman et al. 2020).

6.1.2 Nanometals

Metal nanoparticles such as silver, copper, and zinc have been studied for their potential antimicrobial properties and ability to increase plant nutrient uptake. When applied correctly, nanoparticles can help mitigate microbial infections and improve nutrient absorption in microgreen legumes, leading to healthier plants and higher yields (Jalal et al. 2019).

6.1.3 Carbon Nanotubes (CNTs)

CNTs are cylindrical nanostructures composed of carbon atoms. When incorporated into growth substrates or coatings, CNTs can increase water and nutrient retention, promote root growth, and even facilitate the delivery of growth promoting substances to plant cells in microgreen legumes (Singh et al. 2018).

6.1.4 Nanofertilizers

Nanofertilizers are nanoparticles designed to deliver essential nutrients to plants in a controlled manner. These nanoparticles can improve nutrient availability and uptake efficiency, leading to increased growth and yield of microgreen legumes. Examples include nano versions of essential plant nutrients such as nitrogen, phosphorus and potassium (Liu and Lal 2015a, b, c; Chen et al. 2021).

6.1.5 Nanoporous Materials

Nanoporous materials are characterized by a network of nano-sized pores that can efficiently store and release water and nutrients as needed by plants. Incorporating nanoporous materials into growing media or hydroponic systems can improve water and nutrient management, resulting in healthier and more vigorous growth of microgreen legumes (Li et al. 2019a, b).

6.1.6 Nanocapsules

Nanocapsules are nanoparticles with a hollow core that can encapsulate active ingredients such as nutrients, pesticides or growth regulators. By encapsulating and protecting these substances, nanocapsules can increase their stability and efficacy when applied to microgreen legumes, leading to improved growth, health and yield (Mozafari et al. 2005).

6.2 Nanoemulsions

These emulsions, where one liquid is dispersed in another at the nanoscale, offer a new way to deliver essential plant nutrients, herbicides or insecticides. The increased nanoscale surface area enables controlled release and improved efficacy compared to conventional formulations.

6.2.1 Nutrient Nanoemulsions

Nanoemulsions can encapsulate essential nutrients such as vitamins, minerals and growth promoting compounds. These nanoemulsions are designed to deliver nutrients directly to the roots or leaf surface of microgreen legumes, ensuring efficient absorption and utilization by plants (Liu et al. 2015).

Nanoemulsions can also serve as carriers for pesticides and herbicides used in pest and weed control in microgreen legume cultivation. These nanoemulsions facilitate the targeted delivery of active substances, increase their effectiveness, and at the same time reduce the impact on the environment and minimize off-target effects (Wang et al. 2020).

6.2.2 Antioxidant Nanoemulsions

Antioxidant compounds encapsulated in nanoemulsions can help protect microgreen legumes from oxidative stress induced by environmental factors such as light exposure and temperature fluctuations. These nanoemulsions can contain antioxidants derived from natural sources such as plant extracts or synthetic antioxidants formulated at the nanoscale for increased stability and bioavailability (McClements and Rao 2011).

6.2.3 Growth Regulator Nanoemulsions

Nanoemulsions can encapsulate growth regulators such as cytokinins, auxins and gibberellins, which play a vital role in regulating plant growth and development. By supplying these growth regulators in nanoemulsified form, growers can achieve precise control over plant growth processes and promote desirable traits such as uniformity, vigor and yield in microgreen legumes (Hajimehdipoor et al. 2016).

6.2.4 Essential Oil Nanoemulsions

Essential oils obtained from herbs and spices are known for their antimicrobial, antifungal and insecticidal properties. Nanoemulsions can increase the stability and dispersibility of essential oils, allowing their effective incorporation into microgreen legume culture systems for pest protection and disease control without causing phytotoxicity or residue accumulation (Huang et al. 2017).

6.2.5 Flavour and Aroma Nanoemulsions

Nanoemulsions can encapsulate flavour and aroma compounds extracted from herbs, spices or other natural sources. These nanoemulsions can be used as flavour enhancers or aroma delivery systems to enhance the sensory appeal.

6.3 Nano Coatings

Nano coatings are thin layers of nanoparticles applied to the surface of food products, including legume microgreens, to increase their durability and quality. Nano coatings can provide protective barriers against moisture, gases and pathogens, reducing spoilage and maintaining freshness. These coatings can also improve the mechanical strength of microgreens, prevent wilting and preserve their structure and appearance. By controlling gas exchange and moisture retention, nano-coatings contribute to extending the shelf life of legumes and ensure that their sensory properties remain intact during storage and distribution.

6.3.1 Moisture Retaining Coatings

Nano coatings are designed to increase the water holding capacity of growing substrates or seeds. By incorporating hydrophilic nanoparticles, these coatings help retain moisture around microgreen legume roots, reducing water stress and increasing overall plant vigour (Li et al. 2019a, b).

6.3.2 Pathogen Resistance Coatings

Nano coatings designed to provide resistance against microbial pathogens and pests. These coatings may contain antimicrobial nanoparticles or compounds that inhibit the growth of harmful microorganisms, thereby reducing the risk of disease and pest attack in microgreen legumes (Huang et al. 2014a, b).

6.3.3 UV Protective Coatings

Nano coatings designed to protect microgreen legumes from harmful ultraviolet (UV) radiation. These coatings typically use nanoparticles that absorb or scatter UV light, protecting the delicate tissues of the microgreens from damage caused by excessive sunlight (Hajimehdipoor et al. 2016).

6.3.4 Antioxidant Enhancing Coatings

Nano coatings enriched with antioxidant nanoparticles or compounds that strengthen the antioxidant capacity of microgreen legumes. By scavenging free radicals and mitigating oxidative stress, these coatings help maintain the nutritional quality and shelf life of microgreens (McClements and Rao 2011).

6.3.5 Temperature Regulating Coatings

Nano coatings designed to modulate the temperature around microgreen legumes to ensure optimal growing conditions. These coatings may contain thermoregulatory nanoparticles or phase change materials that absorb or release heat, helping to dampen temperature fluctuations and maintain an ideal environment for growth (Chen et al. 2018).

6.3.6 Nutrient-Release Coatings

Nano coatings designed to gradually release essential nutrients into microgreens over time. These coatings can use nanoparticles to encapsulate nutrients such as nitrogen, phosphorus and micronutrients, ensuring a steady supply of nutrients to support continued growth and development (Liu et al. 2015).

6.3.7 Adhesion-Promoting Coatings

Nano-coatings formulated to improve the adhesion of seeds or growing media to surfaces such as dishes or hydroponic systems. These coatings may contain adhesive nanoparticles or surface-modifying compounds that improve attachment and stability of microgreen legumes during cultivation (Li et al. 2022a, b). The field of nanotechnology is revolutionizing food preservation techniques, presenting a plethora of progressive solutions for extending shelf existence and retaining quality control. At the leading edge of this advancement lies the usage of nanocoatings, microscopic films implemented to the surface of culmination, vegetables, and other perishables.

Nanotechnology offers innovative solutions for extending shelf life and ensuring food safety. This approach utilizes various materials to create functional coatings for food packaging such as:

- **Shelf Life Extension:** Protein-SiOx nanocomposite films, zinc oxide (ZnO) nanoparticle-coated PVC films, and chitosan-based coatings (alone or combined with silicon nanoparticles, nisin, calcium carbonate, or carboxymethyl cellulose) create barriers

that limit gas exchange and moisture transfer, thereby extending shelf life (Liu et al. 2019; Wang et al. 2022a, b; Xu et al. 2018).

- **Quality Control and Decay Restriction**: Coatings containing ZnO nanoparticles, chitosan (with or without additional components like TiO_2, natamycin, or halloysite nanotubes), and alginate-silver nanoparticles can serve as visual indicators of spoilage by changing color or act directly against microbial growth (Liu et al. 2019; Wang et al. 2022a, b; Xu et al. 2018).
- **Antimicrobial Activity**: Incorporation of essential oils (clove or basil) or silver nanoparticles into chitosan-based coatings or edible alginate films combats spoilage microorganisms, enhancing food safety (Liu et al. 2019; Wang et al. 2022a, b; Xu et al. 2018).
- **Emerging Technologies:** Nitric oxide coatings on chitosan nanoparticles and ultrasound-assisted ZnO nanoparticle coatings are promising areas of research for potential applications in quality control and shelf life enhancement.

6.4 Nano Packaging

Nano packaging refers to the use of nanotechnology in the development and implementation of advanced packaging materials and systems for various products, particularly in the food and beverage industry. These packaging materials are engineered at the nanoscale, typically using nanoparticles and nanocomposites, to improve the protection, safety and durability of perishable goods. The goal of nano packaging is to create protective barriers that effectively inhibit microbial growth, control gas exchange, and minimize degradation factors such as exposure to moisture and light. Using nanotechnology, nanopackaging offers innovative solutions to extend product freshness and quality, reduce food waste, and meet consumer demands for safer and more sustainable packaging options.

6.4.1 Active Packaging

Active packaging systems contain nanomaterials that actively interact with the packaged food and extend its shelf life. For microgreen legumes, active packaging can include nanosensors that monitor environmental conditions in the package and release compounds that inhibit microbial growth or absorb ethylene gas,

thereby slowing aging (López-Arbeloa et al. 2016a, b; Muñoz-Muñiz et al. 2020).

6.4.2 Nanofibrous Packaging

Nanofibrous materials offer a high surface area to volume ratio and can be produced with controlled porosity and mechanical properties. Nanofiber-based packaging can provide excellent barrier properties against oxygen and moisture while allowing controlled gas exchange, thus preserving the freshness of microgreen legumes (Dutta et al. 2018; Li et al. 2020).

6.4.3 Smart Packaging

Smart packaging systems integrate nanosensors or indicators that stumble on adjustments within the great or safety of packaged ingredients. For microgreen legumes, clever packaging can include nanobiosensors able to detecting microbial infection or temperature adjustments that provide early caution signs of spoilage (Mukherji and Thakur 2006; Huang et al. 2018). Several strategies contribute to extended shelf life and reduced spoilage. Biodegradable films containing thymol create barriers that minimize gas exchange and moisture transfer. Similarly, polyethylene composites containing silver, kaolin, or titanium oxide (Liu et al. 2019) and various low-density polyethylene (LDPE) films incorporating metal oxides achieve the same effect. Additionally, nanocomposite packaging materials incorporating titanium oxide under ultraviolet light, chitosan with titanium oxide (Xu et al. 2018), and nanocomposite packagings with combinations like polyethylene-vanillin nanoparticles and chitosan offer dual functionality. They can act as visual indicators of spoilage or directly inhibit microbial growth (Liu et al. 2019; Xu et al. 2018). Packaging materials containing nanoparticles can serve as quality control mechanisms. Silicon oxide nanocomposites, potassium permanganate-zeolite composites and silver nanoparticle-containing polyethylene (Liu et al. 2019) can potentially change color or exhibit other visual cues to indicate spoilage. Nanoparticles with inherent antimicrobial properties can be incorporated into packaging to enhance food safety. Silver nanoparticles, used alone or combined with other materials (Liu et al. 2019), and natural modified atmosphere packaging with cellulose and silver nanoparticles offer this benefit. Ozone

fumigation with nanofilm packaging is an emerging technology with potential for freshness management and quality control (Table 4).

Table 4 The table explores the use of nanotechnology in microgreen legume products

Title	Focus	Relevance to legume microgreens	Reference
Chitosan nanoparticles: a promising novel drug delivery system for targeted tumor therapy.	Drug delivery using chitosan nanoparticles	While this study focuses on drug delivery for cancer treatment, it highlights the potential of chitosan nanoparticles as carriers. This technology could be adapted to encapsulate essential nutrients for microgreens.	Desai et al. (2017)
Silver nanoparticles as antimicrobial agents: a case study on E. coli as a model organism.	Antimicrobial properties of silver nanoparticles	This study explores the use of silver nanoparticles for their antimicrobial activity. While further research is needed for microgreens specifically, it demonstrates the potential of nanomaterials to combat spoilage-causing microbes.	Sondi et al. (2004)
Nano-biocomposite films with potential for food packaging.	Nanocomposite materials for food packaging	This research investigates the use of clay nanofillers to improve the barrier properties of food packaging films. The findings suggest the potential of nanocomposite materials to create an ideal environment for preserving microgreens during storage.	Fortunati et al. (2007)

Each entry focuses on a specific area of nanotechnology research and its potential impact on improving the cultivation, storage, or nutritional value of microgreens

6.5 Nano Bubble Technology

Nanobubble technology refers to the application of nanotechnology in the creation and use of extremely small gas bubbles, typically tens to hundreds of nanometers in size, in a liquid medium. This technology involves the generation of nanobubbles using specialized equipment and techniques. The purpose of nanobubble technology is to introduce oxygen or other gases into liquids such as water or nutrient solutions to improve various processes in various industries. In agriculture, nanobubble technology is often

used to oxygenate nutrient solutions in hydroponic systems, promoting root health and increasing plant nutrient uptake (Li et al. 2019a, b). In addition, nanobubble technology finds application in wastewater treatment, where the small size and large surface area of the bubbles facilitate efficient oxygen transfer and increase the degradation of organic pollutants (Watanabe et al. 2018a, b). Nanobubble technology offers a versatile and effective approach to improving processes in agriculture, environmental remediation and other fields by exploiting the unique properties of gas bubbles at the nanoscale (Table 5).

Table 5 This table summarizes different ways nanotechnology is being used to improve agriculture

Application	Description	Benefits	Potential drawbacks
Nanofertilizers	Nutrients encapsulated in nanoparticles for targeted delivery to plants (Liu and Lal 2015a, b, c)	Increased nutrient uptake and efficiency Reduced fertilizer waste and environmental impact Improved crop yield and quality	Higher cost compared to conventional fertilizers Potential unknown environmental effects of nanoparticles
Nanopesticides	Pesticides formulated with nanoparticles for controlled release and targeting specific pests (Kah and Hofmann 2014)	More effective pest control with lower overall pesticide use Reduced harm to beneficial insects and the environment Improved crop protection	Similar cost concerns as nanofertilizers Potential development of pest resistance
Nanosensors	Tiny sensors used to detect plant diseases, nutrient deficiencies, and environmental conditions (Gao et al. 2019)	Early detection of problems for improved crop management Precision agriculture practices based on real-time data Reduced use of pesticides and fertilizers	Need for development of user-friendly and cost-effective sensors Limited range of detection capabilities for some sensors
Smart packaging	Food packaging materials with incorporated nanoparticles for enhanced	Extended shelf life of fruits, vegetables,	Potential higher cost of packaging materials

Application	Description	Benefits	Potential drawbacks
	preservation and monitoring (Aday and Sun 2016)	and other agricultural products Reduced food spoilage and waste Real-time monitoring of freshness and safety	Safety concerns regarding potential migration of nanoparticles to food

It compares applications like targeted fertilizers and smart packaging, highlighting their potential benefits and drawbacks

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7 Nanotechnological Interventions for Legume Microgreens

7.1 Enhanced Nutrient Delivery

Nanoparticles act as microscopic carriers encapsulating essential vitamins and minerals (Liu and Lal 2015a, b, c). This targeted approach allows for controlled release of nutrients within microgreens, increasing their bioavailability and overall nutritional profile (Liu and Lal 2015a, b, c). Consumers benefit from microgreens enriched with essential nutrients, making them a more potent dietary source.

7.2 Combating Microbial Growth

Microbial growth, particularly by bacteria and fungi, significantly impacts microgreen freshness. Nanoparticles with inherent antimicrobial properties can be incorporated into coatings applied to microgreens (Huang et al. 2014a, b). These coatings act as protective shields, effectively combating microbial growth and extending the shelf life of microgreens (Huang et al. 2014a, b). Nanotechnology ensures the quality and safety of microgreens by inhibiting spoilage microorganisms.

7.3 Extended Shelf Life

Traditional packaging materials often fail to create optimal storage conditions, leading to premature deterioration of microgreens. Nanotechnology offers a solution through the development of nanocomposite packaging materials (López-Arbeloa et al. 2016a, b). These intelligent materials regulate gas exchange and moisture

levels within the package, creating a microclimate that optimizes freshness and extends the shelf life of microgreens (López-Arbeloa et al. 2016a, b). By maintaining ideal storage conditions, nanotechnology minimizes quality degradation and reduces food waste.

7.4 Genetic Modification to Increase Nutrients

Gene editing techniques like CRISPR-Cas9 allow scientists to target specific genes in microgreens responsible for nutrient uptake and biosynthesis (Kus CRISPR et al. 2023). This approach can increase essential nutrients in microgreens, making them more nutritious and beneficial for consumers (Kus CRISPR et al. 2023). Precise manipulation of the genetic makeup of microgreens enables researchers to tailor their nutritional content to specific dietary needs and preferences.

7.5 Transcriptomic Analysis of Taste Compounds

Transcriptomic analysis allows for identification of genes involved in flavour development in microgreens (Wang et al. 2022a, b). By understanding gene expression patterns related to taste and aroma, researchers can manipulate gene expression to improve the sensory properties of microgreens (Wang et al. 2022a, b). This targeted approach allows for improved flavour profiles, making microgreens more appealing to consumers and expanding their culinary uses.

7.6 Proteomic Profiling for Quality Assessment

Proteomic techniques analyze protein content and post-translational modifications in microgreens, providing insights into their nutritional quality and sensory properties (Hajduch et al. 2017). This analysis assists researchers in evaluating and improving the overall quality of microgreens by identifying key proteins associated with taste, texture, and nutritional value (Hajduch et al. 2017). By understanding the proteomic profile of microgreens, researchers can optimize growing conditions and post-harvest handling practices to ensure consistently high product quality.

The implementation of these nanotechnological advances in the production of microgreen legumes holds significant promise for the agricultural sector. By minimizing spoilage and extending shelf life,

nanotechnology can contribute to reducing food waste associated with microgreens. In addition, ensuring the quality and availability of microgreens can improve dietary diversity and promote access to essential nutrients, especially for vulnerable populations facing food insecurity. The potential environmental and human health impacts associated with the use of nanoparticles in food systems require thorough investigation (Table 6).

Table 6 A comparison of commercial solutions

Company	Commercial materials	Product	Applications (focus)	Forms	References
NanoGrow	Nanotechnology substrates & coatings	NanoGrow solution	Optimize microgreen growth	Substrates & Coatings	Liu et al. (2019)
MicroNanotech	Micro structured nanomaterials	MicroGreen boost	Promote root development & water retention	Hydroponic Systems & Substrates	Wang et al. (2022a, b)
NanoGreens	Nanoparticle-infused fertilizers & growth media	NanoGreens fertilizer	Enhance growth & nutrient absorption	Fertilizers & Growth Media	Xu et al. (2018)
GrowTechNano	Nanotechnology-enabled sensors & monitoring systems	NanoMonitor	Optimize environmental conditions	Sensors & Monitoring Systems	Patil et al. (2016)
NanoAgriTech	Nano-engineered seeds & seed coatings	NanoSeeds	Increase germination rate & enhance plant resistance	Seeds & Seed Coatings	Desai et al. (2017)

This table compares relevant companies offering nanotechnology-based solutions for microgreen production

8 Limitations of Nanotechnology in Leguminous Microgreens and Agriculture

Nanotechnology has emerged as a revolutionary field with enormous potential in agriculture. However, its application in legume microgreens and wider agriculture also presents certain limitations that require careful consideration. Here is a breakdown of those restrictions, along with citations:

8.1 Uncertain Impacts on Plant Physiology and the Microbiome

Nanoparticles can interact with plant tissues in unpredictable ways, potentially affecting their growth, nutrient uptake and overall health (Colvin et al. 2019a, b; Liu and Lal 2015a, b, c). The impact of nanoparticles on the complex rhizospheric microbiome (the community of microorganisms surrounding plant roots) remains largely unexplored, and unintended disruption could have negative consequences for plant health and nutrient cycling (Liu et al. 2019).

8.2 Lack of Long-Term Safety Data

The long-term effects of the release of artificial nanoparticles into the ecosystem on the environment and human health are not fully understood (Colvin et al. 2019a, b). Research is ongoing to assess the potential risks associated with the persistence of nanoparticles in soil, their potential entry into the food chain and potential toxicity issues (Khattak et al. 2017).

8.3 Regulatory Challenges

Existing regulatory frameworks may not be adequately equipped to address the unique risks and uncertainties associated with nanotechnology in agriculture (Chen et al. 2015a, b). Establishing clear guidelines for the development, testing and commercial use of nanomaterials in food production is essential to ensure safety and consumer confidence (Gupta and Xie 2018a, b).

8.4 Cost and Scalability

- The synthesis and application of nanoparticles can be costly and energy intensive, limiting their economic viability for widespread adoption in agriculture, especially for smallholder farmers.
- Scale-up of nanotechnology for large-scale agricultural applications remains a challenge and cost-effective production methods need to be developed.

8.5 Ethical Aspects

The potential unintended consequences of introducing engineered nanoparticles into the environment raise ethical concerns that require careful consideration.

9 Conclusion

This chapter explores the potential of nanotechnology to solve problems related to the production and storage of legume microgreens. Although microgreens are nutritious foods, they have a short shelf life due to their high metabolic activity and rapid perishability. Nanoparticle-based packaging materials have enhanced barrier and anti-bacterial properties, which can reduce moisture, oil exchange, and microbial contamination, thus extending the shelf life of microgreens. Nanocoatings applied directly to microgreens can provide additional protection against these damages and enhance their quality and visual appearance. Nanotechnology offers new solutions such as nanobubble technology, which creates an oxygen-rich environment and inhibits microbial growth, potentially reducing the need for harsh chemicals in microgreen production. The use of nanoparticles with antimicrobial properties is expected to control the microbial activity of microgreens, as well as help extend shelf life and improve safety. However, more research is needed to ensure the safety and effectiveness of these new applications and to thoroughly evaluate their environmental impacts. Nanotechnology has great potential for the future of the micromung bean industry, allowing producers to deliver fresh, high-quality micromung beans to consumers while also supporting food sustainability. The development of nanotechnology brings great promise for legume microgreens and offers a way to increase growth, quality and durability through the novel use of nanomaterials in non-agricultural soils. Through the use of nanotechnology, legume microgreens can enhance absorption using targeted delivery using nanoparticles and enhance immunity using nanomaterials in antibiotic diseases. Holistic development in plant nanotechnology leads to health by promoting healthy growth. In addition, nanotechnology can also create new delivery systems for nutrients and bioactive compounds to improve the nutrient composition and performance of legume microgreens. The effect of nanotechnology on legumes has important implications in terms of quality improvement, shelf life extension and food safety. However, responsible development and research is essential to ensure the long-term benefits and sustainability of this technology in the microgreen legume industry.

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Engineered Nanoparticles for the Betterment of Legume Microgreens: A Detailed Overview

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1 Introduction

Legume plant belongs to the family Leguminaceae or Fabaceae, unique for their flower pattern, and their seeds arranged in a pod (Katoch and Katoch 2020). Legume grains are the second most consumed human dietary products worldwide. Mainly legume serves two types of food sources—an animal-free protein source and an oil source (Shevkani 2023). Other than the legume seeds the young seedling and twig portion of some legume plants are consumed as leafy vegetables, known as microgreens. Because of their high nutritional properties, these microgreens are being incorporated into the food palate of all communities worldwide (Darai et al. 2023). In the present times, the production of LMGs is facing various kinds of environmental obstacles due to rapid urbanization and

greenhouse gas emissions. The environmental obstacles include various abiotic (salinity, drought, high temperature, chilling, heavy metal contamination, flood) and biotic (parasites, pests, fungus, bacteria, virus infection) factors, that are causing a drastic impact on total production (Dutta et al. 2022; Rane et al. 2021). Due to these stresses, a 100% loss in production occurs very often, other than that the nutritional properties and the quality of the products also get affected (Sarkar et al. 2021).

To overcome these stresses, in the past decade, the potential of nanotechnology has been explored. The use of nanoparticles in all possible ways is not only found to mitigate those stress effects but also aid in the improvement of production and nutritional values of legume plants (Elemike et al. 2019; Zhao et al. 2020). For example, the use of calcium phosphate nanoparticles has been found to improve the productivity of saline-stressed broad bean plants along with improved growth, photosynthetic attributes, antioxidant defense, and proline and phenol accumulation (Nasrallah et al. 2022). Similarly, the negative effects of drought stress on pea plants have also been found to be mitigated through the application of silica nanoparticles, which improved the relative water content, shoot growth, antioxidant defense, and phenolic accumulation in the plants (Sutulienė et al. 2021). The application of silica nanoparticles has also been found to improve the growth and health of *Phaseolus vulgaris* plants growing under heavy metal-contaminated saline soil. Using the nanoparticles not only improved plants' growth, photosynthesis, antioxidant defense, and osmotic status but also reduced heavy metal accumulation in the aerial parts significantly (El-Saadony et al. 2021). Besides the abiotic stress amelioration, the use of silica nanoparticles (seed priming) alone or in combination with *Rhizobium leguminosarum* has been observed to reduce the infection rate of *Meloidogyne incognita* and *Pseudomonas syringae* pv. *pisi* on *Pisum sativum* (Kashyap and Siddiqui 2021). Moreover, the biosynthesized silver nanoparticles have also been found to provide resistance to the faba bean plants against yellow mosaic virus (Abdelkhalek et al. 2022).

Moreover, the efficiency of nanoparticles can be increased by modifying the nanoparticles in different ways, and has been referred to as engineered nanoparticles. For the synthesis of engineered nanoparticles, their surface can be modified with some bioactive molecules, entrapment or encapsulation by other nanoparticles, and internalization of other nutrients/substances in their pores (Dimkpa et al. 2012; Shakiba et al. 2020; Singh et al. 2021a, b). These engineered nanoparticles are more efficient than bare nanoparticles to combat abiotic or biotic stresses. For example, sugar-functionalized carbon nanotubes have been found to reduce the salinity stress in *Vigna radiata* seedlings along with better growth and physiology compared to bare carbon nanotubes (Sarkar et al. 2022). In agreement with this, the trehalose functionalized silica nanoparticles have been observed to be more efficient than the bare silica nanoparticles in the betterment of lentil seedling growth and development in presence of salinity stress (Sarkar et al. 2024).

Thus this chapter aims to discuss the detailed efficacy of nanoparticles and engineered nanoparticles in the growth and nutritional aspects of LMGs. Additionally, the capability of the nanoparticles and engineered nanoparticles to mitigate various abiotic and biotic stresses of LMGs is also discussed along with the molecular regulations and phytohormone signaling conferring greater stress tolerance.

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2 LMGs and Their Nutritional Properties

Plants are considered microgreens in their young seedling stage grown between 7–28 days, when the whole shoot portion is edible, but not roots (Di Gioia et al. 2017). Some well-known LMGs include *Lens culinaris*, *Pisum sativum, Cicer indicum, Cicer arietinum, Vigna radiata, Vigna unguiculata, Onobrychis sativa, Bilensoy clover, Medicago sativa,* and *Trifolium pratense* (Altuner et al. 2022; Lone and Pandey 2024). These LMGs are highly nutritious consisting of high protein, carbohydrate,

mineral, and fiber content. Except for these, they are high sources of secondary metabolites, antioxidants, and phytochemicals (Butkute et al. 2018; Table 1). Some of the important secondary metabolites present in LMGs include triterpene saponins, phytoestrogens, and condensed tannins which have various medicinal properties like anti-diabetic, anti-inflammatory, anticancer, antimenopause, antihelmintic, cardioprotective, etc. (Butkute et al. 2018). LMGs can be consumed as vegetables to make curry and soup, salads, or integrated into different types of bread to increase their nutritional properties (Klopsch et al. 2018; Rai et al. 2022). The LMGs are being used as food traditionally in various parts of the world. High nutritional properties make them acceptable as mainstream food sources (Butkute et al. 2018).

Table 1 Nutrient profile of some of the frequently used LMGs

Common name	Scientific names	Nutritional Content	References
Soybean	*Glycine max*	Proteins, lipid, carbohydrate, rich in phenolics (isoflavone, phenolic acid, and flavonol)	Zhang et al. (2019)
Fenugreek	*Trigonella foenum-graecum*	Protein, dietary fibres, ascorbic acid, magnesium, phosphate, β-carotene	Ghoora et al. (2020)
Chickpea	*Cicer arietinum*	Carbohydrate, protein, total phenol, amino acid, tannin, antioxidants	Kurian and Megha (2020)
Bilensoy alfalfa	*Medicago sativa*	Rich in antioxidants and flavonoids	Altuner (2021)
Lentil	*Lens culinaris*	Proteins, minerals, total phenolics, total carotenoids, ascorbic acid, peroxidase, DPPH, FRAP	Priti (2021)
Mung bean	*Vigna radiata*	Proteins, minerals, total flavonoid, total amino acids, ascorbic acod, peroxidase, catalase, DPPH	Priti (2021)
Black gram	*Vigna mungo*	Protein, flavonoids, phenolics, antioxidants	Kour et al. (2022)
Alfalfa	*Medicago sativa*	Carotenoid and antioxidant rich	Fabek Uher et al. (2023)
Green Pea	*Pisum sativum*	Proteins, phenolic compounds, vitamins, antioxidants, and macro-	Garcia-Tenesaca et al. (2024)

Common name	Scientific names	Nutritional Content	References
		and microelements	

3 Factors Affecting the Nutrient Profile of LMGs

Being a high source of nutrients, LMGs are being considered as a superfood for the near future. These nutrient contents can be affected by different growth factors like growing substrate/ medium, light, temperature, altitude, environmental stresses, and seed priming (Ebert 2022). For example, microgreens of mung bean, black gram, and chickpea were found to have better nutrient profiles (protein, flavonoids, phenolics, and antioxidants) while grown on cocopeat with the nutrient solution as compared to the soil-grown microgreens irrigated with normal water (Kaur et al. 2022). Additionally, the higher altitude (Leh) grown mung bean and lentil microgreens were found to be superior in nutrient profiles (ascorbate, tocopherol, flavonoid, carotenoids, phenolics, antioxidants (DPPH and ABTS scavenger), micro, and macronutrients contents) as compared to the lower altitude grown microgreens. These could be due to the presence of high photosynthetically active radiation, wide temperature amplitude, and UV-B which makes the weather of Leh unique compared to others (Priti et al. 2021). Additionally, light intensity and irrigation regimes also have a direct role in the growth of LMGs. In this connection, the *Vigna radiata* microgreens were found to be healthier when grown under growth light (LED) and 60 mL/day water routine as compared to the room light exposed and 10 mL/day water routine grown microgreens (Manickavasagan 2021). Similarly, the intensity and type of light exposure are directly related to the polyphenol content of legume sprouts. For example, 11 W/m^2 photosynthetic (red LED) light-grown and 1 W/m^2 stress (UV-A) light-grown lentil seed sprouts were found to have enhanced antioxidant activities and phenolic (luteolin, ferulic acid, tricetin, and kaempferol) content as compared to

the blue light-grown and UV-B light grown lentil sprouts respectively (Park et al. 2024). Moreover, the blue, red, and white light-grown mung bean microgreens were found to have increased amounts of polyphenols (caffeic acid, genistin, rutin, vitexin, and delphinidin 3-glucoside) compared to the dark-grown microgreens. Additionally, plant height was found to be the highest in blue light-grown mung bean microgreens followed by red light and white light-grown plants (Cheng et al. 2023).

On the other hand, seed-priming is one of the key factors that can affect germination potential, as well as nutritional attributes. For example, seed nutri-priming with different Zn sources was found to improve the Zn content in pea and sunflower microgreens along with the improvement in total yield and phytochemical components (Poudel et al. 2023). Similarly, potassium nitrate priming as a nitrogen source was found to improve the biomass accumulation in the microgreens of *Medicago indicus, Medicago interexta,* and *Medicago polymorpha.* Along with the improved biomass, increment in mineral, vitamin, pigment, amino acid, phenolic, and flavonoid contents, antioxidants, and antidiabetic activities have also been found (Zrig et al. 2021). In agreement with this, molybdenum ion pre-treatment resulted in increased growth and biomass of the *Canavalia* species (*Canavalia ensiformis* var. *gladiata*, *Canavalia ensiformis* var. *truncata*, and *Canavalia gladiata* var. *alba*), along with improved nitrogen and pigment content and increased antioxidant activities (Okla et al. 2021). In order to improve the microgreens' antioxidant and secondary metabolite profile, they have been intentionally introduced to some stress factors (stress light, temperature, salinity, metal treatment, phytohormones). The reason behind this is to expose the seedlings to stressors for a certain time which help the microgreens to biosynthesize the antioxidants and secondary metabolites as defense factors that ultimately improve the microgreen quality (Zielińska-Dawidziak 2021).

4 Recent Advances in the Application of Nanoparticles for the Improvement of LMGs

Nanotechnology is an excellent emerging technique that has been explored successfully for the betterment of agricultural development in the last one and a half decades. Several studies have confirmed that the nanoparticles has a direct role in the improvement of crop health (Singh et al. 2021a, b; Table 2). Nanoparticles are tiny particles ranging between 1 and 100 nm in size (at least in one dimension), with a high surface area by volume ratio, making them more efficient than their bulk materials (Chandraker et al. 2022). Being tiny in size they can be easily uptaken by plants which helps the plant to have better growth and development (Elemike et al. 2019). Some of the important nanoparticles that have been explored to have the best efficiency in improving crop health include silica nanoparticles, carbon nanoparticles, zinc oxide nanoparticles, silver nanoparticles, chitosan nanoparticles, titanium dioxide nanoparticles, iron nanoparticles, etc. (Saleem and Zaidi 2020). These nanoparticles were studied to have a great role in the improvement of growth and nutrient quality of LMGs also. For example, silica nanoparticles have been observed to increase the germination attributes, shoot-root growth, and dry mass of *Phaseolus vulgaris* microgreens compared to the untreated ones (Alsaeedi et al. 2017). Presoaking of *Vigna radiata* seeds in the solution of zinc oxide nanoparticles has been found to improve the germination indices, growth, and biomass accumulation of the microgreens compared to the dH_2O-treated ones (Jayarambabu et al. 2015). Along with the better growth, the protein, pigment, and antioxidants were found to improve in 4-day-old microgreens of *Vigna radiata* while applied with carbon nanoparticles (Shekhawat et al. 2021). In agreement with this, the application of silver nanoparticles has been found to improve the germination attributes and fresh and dry weight of fenugreek (*Trigonella foenum-graecum*) microgreens compared to the untreated seedlings (Hojjat and Hojjat 2015).

Similarly, the application of selenium nanoparticles individually and/or in combination with lentinans (a mushroom-derived polysaccharide) has been observed to improve the pea seedling (microgreen) biomass, amino acid, and polyphenol content (Lin et al. 2023). Moreover, seed priming with selenium nanoparticles and β-amino butyric acid (BABA) was found to increase the growth and pigment content in *Medicago interexta* microgreens, along with additional improvement in essential amino acids, secondary metabolites, mineral contents, antioxidant, and anti-diabetic activities (Selim et al. 2022). Additionally, mixed metal-based (Co, Fe, Zn, Mo, Mn, Cu, and Se) nano priming of different pea cultivars seeds showed improved germination parameters along with improved seedling growth, biomass, and pigment contents when compared to the hydroprimed and non-primed seeds (Tamindžić et al. 2023). Other than the chemically synthesized nanoparticles, a plant-based nanoformulation using Triphala (*Terminalia bellirica, Terminalia chebula* and *Emblica officinalis*) extract has also been found to improve the shoot-root lengths and biomass, pigment content along with improved protein and carbohydrate in the microgreens of *Vigna radiata* and *Trigonella foenum-graecum* (Ranjani and Hemalatha 2023). The schematic representation of the beneficial properties of nanoparticles have been presented in Fig. 1.

Table 2 Recent reports of the efficacy of bare and functionalized nanoparticles in the growth, and stress alleviation of LMGs

Nanoparticle/Engineered nanoparticles applied	Host legume microgreen (seedling)	Purpose of application	Effects after application	References
Zinc and Iron nanoparticles	Pigeon pea (*Cajanus cajan*)	Seed polymer coating	Increased seed germination, seedling length, seedling dry weight, field emergence, seedling vigour; Increased	Korishettar et al. (2016)

Nanoparticle/Engineered nanoparticles applied	Host legume microgreen (seedling)	Purpose of application	Effects after application	References
			dehydrogenase activity and amylase activity	
Acylated homoserine lactones loaded magnetic carbon nanofibers	Chickpea (*Cicer arietinum*)	Nano-composite fertilizer	Increased biomass, chlorophyll, and protein contents	Gupta et al. (2019)
Glucose, levan, and pullulan coated cerium oxide nanoparticles	Pea (*Pisum sativum*) and wheat	Metal stress	Increased seed germination, seedling growth, and resistance against stress through improved total antioxidant activities and total phenolic content	Milenković et al. (2019)
Bacillus subtilis encapsulated in titanium nanoparticles rich alginate– bentonite coating	Beans (*Phaseolus vulgaris* L.)	Defense against *Rhizoctonia solani*	Enhanced resistance against the pathogen through higher production of indole-3-acetic acid; Improved growth parameters	Saberi-Rise and Moradi-Pour (2020)
Iron nanoparticles + *Rhizobium* inoculation	Common bean (*Phaseolus vulgaris*)	Fertilizer	Increased plant growth, and nodule formation; Enhanced symbiotic nitrogen fixation	De Souza-Torres et al. (2021)
Silica and carbon nanoparticles enriched alginate/ gelatin	Common bean (*Phaseolus vulgaris*)	To control *Rhizoctonia solani*	Improved plant growth, and resistance	Moradi-Pour et al. (2021)

Nanoparticle/Engineered nanoparticles applied	Host legume microgreen (seedling)	Purpose of application	Effects after application	References
encapsulated *Bacillus velezensis*			against pathogen	
Gibberellic acid (GA_3) and Indole acetic acid (IAA) loaded polyvinyl alcohol nanofiber	Peanut (*Arachis hypogaea*) and Black gram (*Vigna mungo*)	Higher moisture stress	Increased seed germination and seedling vigour	Raja et al. (2021)
Silver nanoparticles	Common bean (*Phaseolus vulgaris*)	Growth inducer	Increased germination and seedling growth	Savassa et al. (2021)
Magnesium nanoparticles	Mung bean (*Vigna radiata*)	Resistance against *Fusarium solani*, and *Fusarium oxysporum*	Improved pathogen resistance; Increased shoot and root growth, fresh and dry weight	Abdallah et al. (2022)
Biosynthesized hematite nanoparticles	Fenugreek (*Trigonella foenum-graecum*)	Drought stress alleviation	Alleviated drought stress through increased nitrogen metabolism (nitrate reductase, glutamine synthetase, and glutamate synthase) and biological nitrogen fixation	Bisht et al. (2022)
Zinc oxide nanoparticle	Chickpea (*Cicer arietinum* L.)	Resistance against *Fusarium oxysporum*	Increased shoot and root growth and dry weight; enhanced resistance against pathogen through	Farhana et al. (2022)

Nanoparticle/Engineered nanoparticles applied	Host legume microgreen (seedling)	Purpose of application	Effects after application	References
			enhanced quantities of sugars, total proteins, phenol, and superoxide dismutase	
Multi-nutrients loaded electrospun PVA nanofiber	Mung bean (*Vigna radiata*)	Growth inducer	Increased seed germination and post-germination growth	Mohanraj et al. (2022)
Carbon dots	*Glycine max*	Drought stress	Improved growth; Increased root exudates attracted rhizobacteria that improved the nitrogen fixation	Wang et al. (2022)
Inulin-coated Zinc oxide nanoparticles	Faba bean (*Vicia faba* L)	Growth inducer	Increased seed germination and post-germination growth through stem cell miosis	Carbone et al. (2023)
Iron nanoparticles	Alfalfa (*Medicago sativa* L.)	Fertilizer	Improved growth, leaf chlorophyll content and photosynthesis rate; Increased nitrogen fixation and nodule development	Zhang et al. (2024)

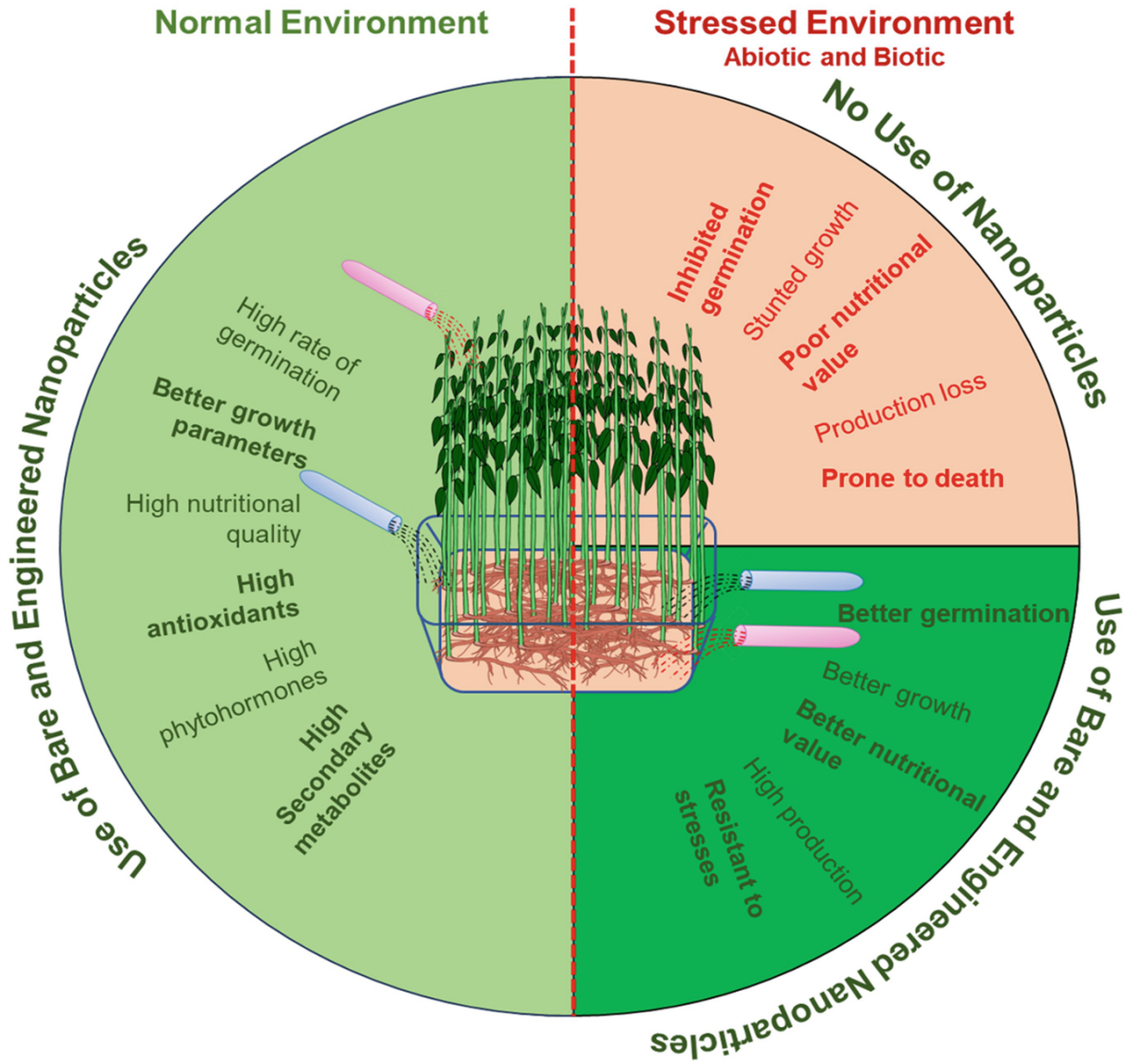


Fig. 1 Beneficial properties of nanoparticles in leguminous microgreens

5 Engineered Nanoparticles in the Improvement of LMGs

Engineered nanoparticles are mainly used in the agricultural sectors for the preparation of slow-release fertilizers and pesticides (Vejan et al. 2021; Vermoesen et al. 2024; Table 2). These engineered nanoparticles increases the bioavailability, and longevity of nanoparticles as well as the bioactive molecules which ultimately improved crop health more

profoundly (Harish et al. 2022). The engineered nanoparticles have already been explored to improve the legume seedling (microgreen) growth and physiology, however, there are only a few reports on the same. For example, the application of citric acid-coated iron nanoparticles has been found to improve the plant growth of soybean and alfalfa, but the translocation of iron to the aerial parts was negligible. Thus it can be said that the iron nanoparticle helps the plant in the better uptake of citric acid, which plays the main role in improving plant growth (Iannone et al. 2021). Moreover, the curcumin-functionalized copper nanoparticles have been observed to improve the germination and growth attributes of chickpea seedlings (Sathiyabama et al. 2020). Song et al. (2022) observed better photosynthetic attributes and antioxidant enzyme activities in alfalfa (*Medicago sativa*) seedlings while applied with silver nanoparticles, whose surface was capped with alfalfa extract. Also, the capping and light induction to the nanoparticle was found to reduce its toxic effects on plants.

Other than surface functionalization, nanoencapsulation of bioactive compounds also has great potential to improve the health and nutritional quality of legume seedlings/microgreens. In this context, thiamine-encapsulated chitosan nanoparticles have been found to improve seed germination and growth of chickpea seedlings along with improved auxin content and defense system against pathogens (Sathiyabama et al. 2019). Similarly, the silver-chitosan nanoparticles have been found to improve the germination attributes of chickpea seeds along with improved seedling growth, dry mass, pigment content, and enzymatic activities (Anusuya and Banu 2016). Moreover, NPK-loaded carbon nanotubes and chitosan nanoparticles were found to be used as a slow-release fertilizer, which ultimately facilitated their uptake through the roots of French bean seedlings. These nanofertilizers improved the water and nutrient uptake in plants and simultaneously enhanced their growth (Hasaneen et al. 2016). Similarly, the urea-loaded chitosan nanoparticles have been observed to improve the height, fresh and dry weight, photosynthetic pigment content, protein, and phenol content of *Vigna radiata* seedlings, which

was even higher compared to bare nano urea application (Sharma et al. 2022). The schematic representation of the beneficial properties of engineered nanoparticles has been presented in Fig. 1.

6 Nanotechnology in the Alleviation of Abiotic Stresses in the LMGs

As described earlier, controlled exposure to abiotic stress improves microgreens' nutritional qualities, which has been reflected by the higher production of osmolytes and secondary metabolites in response to controlled stress conditions. However, the higher intensity of stresses drastically affects the microgreen's growth and development (Fig. 1; Araujo et al. 2015). The increasing population and rapid urbanization reducing the fertile croplands, and the rising environmental pollution creates several kinds of abiotic stresses that drastically affect crop production (Hossain et al. 2020). In this situation, the development of sustainable food security demands improvisation in farming techniques in such a way that crop production can be improved without causing much damage to the ecosystem (Sjah and Zainuri 2020). In this connection, the use of nanoparticles have shown great efficiency in improving the growth and development of crops under stressed conditions. There are many reports on the beneficial role of nanoparticles in improving the growth and health of legume seedlings (microgreens). For example, seed priming with silica nanoparticles has been observed to alleviate the salinity stress of *Vicia Faba* microgreens with improved growth parameters (Aboellail et al. 2023). Similarly, the titanium dioxide nanoparticles have been found to improve the germination indices and growth attributes of *Lathyrus sativus* seedlings in the presence of salinity stress (Hojjat et al. 2020). Silver nanoparticles have also been found to improve the germination indices and growth of lentil seedlings under drought-stress conditions (Hojjat and Ganjali 2016). Also the pre-sowing application of silver nanoparticles along with

Rhizobium leguminosarum bv. *phaseoli* has been observed to improve the *Phaseolus vulgaris* seedling growth, biomass, and photosynthesis in the presence of chilling temperature (Prazak et al. 2020). Again the combined application of iron and silica nanoparticles has been found to reduce the cadmium stress in *Phaseolus vulgaris* seedlings by inducing polyamine biosynthesis (Koleva et al. 2022).

Engineered nanoparticles have also been observed to alleviate different abiotic stresses (Table 2). For example, seed nano-priming with cysteine-functionalized zinc oxide nanoparticles was found to confer better germination attributes, seedling height, dry mass, hydrolytic and antioxidant enzyme activities in *Vigna radiata* microgreens under water stress conditions while compared to the unprimed seeds (Kalimuthu et al. 2023). Similar improvements in seedling growth, biomass accumulation, and pigment content were found in saline-stressed *Vigna radiata* varieties (NM-92 and AZRI-2006) when the seeds were nano-primed with glutamic-acid functionalized iron nanoparticles (Ul Haq et al. 2023). Moreover, the zinc nanoparticles loaded with *Moringa oleifera* extract have been found to increase the shoot/ root length and biomass, photosynthetic pigment, and mineral content of *Vicia faba* in the presence of salinity (Ragab et al. 2022).

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7 Nanotechnology in the Alleviation of Biotic Stresses in the LMGs

During the germination and early growth period of legumes, they are also prone to several pathogen and pest attacks. Thus, the seedlings' germination attributes and growth are severely affected (Lamichhane et al. 2018). Nanotechnology in this context has displayed tremendous potential to negate the harmful effects of such pathogens and pests (Table 2 and Fig. 1). For instance, chitosan nanoparticles were found to reduce the infection of *Fusarium oxysporum* f. sp. *ciceris* (a soil-born fungal pathogen that can lead to 100% crop loss) in chickpea

plants in different life stages of the plants under variable environmental conditions (Sravani et al. 2023). Another soil-borne disease known as root rotting caused by *Rhizoctonia solani* has also been found to be reduced in both early (microgreen) and late stage of fava bean crops life when treated with selenium nanoparticles biosynthesized from *Bacillus megaterium* (Hashem et al. 2021). Similarly, the *Phaseolus vulgaris* plant showed greater resistance against early and late damping-off resulting from root rot fungus (*Pythium graminicola*, *Rhizoctonia solani*, *Macrophomina phaseolina*, and *Sclerotium rolfsii*), when treated with silver nanoparticles. Along with the resistance against fungus, the silver nanoparticles were found to improve the seedling vigour, seedling height, and dry mass (Ibrahim et al. 2024). Combined and individual treatment of chitosan and silver nanoparticles have also been observed to reduce the infection rate of *Botrytis fabae* (chocolate spot disease) and *Alternaria alternata* (*Alternaria* leaf spot disease) by 50% in fava bean leaves (Ahmed 2017). Similarly, the combination of selenium and silica nanoparticles has been observed to impart similar resistance as traditional fungicides against *Alternaria alternata* fungus that causes leaf spot disease in the *Phaseolus vulgaris* plants (Taha et al. 2023). Another combined bimetallic nanoparticle of zinc and boron was found to have excellent antifungal activities against *Pythium irregulare* a soil-borne pathogen of pea plant (Elkhodary et al. 2023).

Along with defending the pathogenic attack, nanoparticles have also been observed to reduce the pest attack in legume plants. For example, the biosynthesized MgONPs were found to increase the death rate of *Callosobruchus maculatus* (a seed storage insect) which ranged from 83% to 100%. Additionally, the same nanoparticles had beneficial roles in the growth and development of the *Vigna unguiculata* plants (Abdelfattah et al. 2023). Similarly, the application of biogenic nickel oxide nanoparticles (5–20 ppm) showed reduced fecundity and increased developmental stage durations of pulse beetle (*Callosobruchus maculatus*) along with improving seed

germination of *Vigna mungo* (Rahman et al. 2021) Thus these nanoparticles can be used in the early stage of legume crop growth to combat the pathogenic and pest attack.

Although there are several studies on the improvement of LMGs using nanoparticles, but very few studies have evaluated the capacity of engineered or modified nanoparticles on legume crop improvement under the presence of biotic stresses. Among the modified nanoparticles, the thymine-loaded chitosan nanoparticles have been found to have great inhibition effects against a soil-born dry root rot disease-causing fungus *Rhizoctonia bataticola* that has detrimental effects on chickpea plants (Sathiyabama et al. 2022). Moreover, the functionalization of copper nanoparticles with curcumin has also been observed to protect chickpea plants against a detrimental pathogen *Fusarium oxysporum* f. sp. *ciceri* (Sathiyabama et al. 2020). These studies clearly showed the excellent potential of modified nanoparticle to combat with pathogenic attack. Thus in near future these modified nanoparticles should be explored in context to reduce the pathogenic as well as pest attack on crop plants.

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8 Crosstalk of Nanoparticles with Phytohormones and the Molecular Background of Their Beneficial Role in LMGs

Phytohormones like auxin, abscisic acid, jasmonic acid, and salicylic acid (SA) have excellent roles in stress-responsive signaling (Parwez et al. 2022). For example, thymine-loaded chitosan nanoparticles were also found to enhance the biosynthesis of indole acetic acid and defense enzyme activities to combat the proliferance of *Fusarium oxysporum* f. sp. *cicero* (Sathiyabama et al. 2020). Another phytohormone-mediated (high accumulation of salicylic acid and jasmonic acid) resistance against *Acyrthosiphon pisum* (aphid) in *Vicia faba* has been induced by the application of chitosan-coated

mesoporous silica nanoparticles, which was even better than the traditionally used acetamiprid pesticide (Ji et al. 2023). Similarly, hollow mesoporous silica nanoparticles application has also been found to induce SA-mediated systemic acquired resistance against *Fusarium oxysporum* f. sp. *phaseolus* infection in cowpea plants. This was evident through the overexpression of SA marker genes *PR-1* and *PR-5* which ultimately improved the seedling growth and biomass (Ding et al. 2024). Similarly, pathogenesis-related (*PR1*) genes have been upregulated when applied with chitosan nanoparticles, to induce resistance against bean yellow mosaic virus in fava bean plants along with improvement in other defense-related properties like total phenol (El Gamal et al. 2022). Silica nanoparticles have also been found to increase the plant volatiles in herbivore (*Spodoptera littoralis*) induced soybean seedlings to attract the natural enemy (rove beetle—*Paederus fuscipes*) of the pest (Thabet et al. 2021). Again the silica nanoparticles have been observed to synthesize high amounts of salicylic acid in *Ralstonia solanacearum infected* peanut plants by upregulating the SA-metabolism gene (*salicylate carboxymethyltransferase*), which is directly involved in systemic acquired resistance (SAR; Deng et al. 2024).

Besides the phytohormone-mediated stress defense mechanism, the activity of bare nanoparticles and engineered nanoparticles in improving the growth of LMGs or leguminous plants relies on the molecular adjustments and fine-tuning of the signaling process that helps the plants combat environmental stresses. To adapt to a stressed environment, plants activate their antioxidant system, and accumulate several secondary metabolites and stress-responsive bioactive molecules (Chen et al. 2022). A plethora of work has been done by several workers to assess the role of nanoparticles in this aspect. In one of the studies, silica nanoparticles were found to improve the antioxidant defense system (superoxide dismutase, ascorbate peroxidase, peroxidase, catalase, and lipoxygenase) that ultimately reduced membrane damage and ROS accumulation in peanut plants in the presence of wilt-causing

bacteria *Ralstonia solanacearum* (Deng et al. 2024). Additionally, the chitosan-coated mesoporous silica nanoparticles have also been found to increase antioxidant activities along with improved defense-related metabolites (coumarin, quercetin) to induce resistance against aphids in *Vicia faba* (Ji et al. 2023). Moreover, the silica nanoparticles have been found to increase the photosynthetic rate of *Pisum sativum* during being infected with a broomrape-causing parasite *Phelipanche aegyptiaca.* Improved photosynthetic rate simultaneously increased the sugar metabolism, and the biosynthesis of amino acids and fatty acids. Most importantly, pretreatment with silica nanoparticles increased the phenylalanine involved in lignin biosynthesis which creates a barrier against the pathogen infestation (Shabbaj et al. 2021). A similar high deposition of lignin was found in the vascular bundles of *Fusarium oxysporum* f. sp. *cicero* exposed chickpea stem while treated with thiamine-encapsulated chitosan nanoparticles. Higher accumulation of antioxidants and phenylpropanoids also provided resistance against the pathogen (Sathiyabama and Indhumathi 2022).

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9 Conclusion

LMGs are beneficial to human health, being rich in protein, vitamin, mineral, and fibre. It provides beneficial secondary metabolites, antioxidants, and phytochemicals that provides the nutrition requrement and therapeutic properties. As a result of this, their demand is increasing day by day, which ultimately increased the interest towards cultivating these microgreens in the recent years. However, the increasing demand could be fulfilled only when their production is enhanced at a gradual pace and losses due to environmental stress are reduced. Because, most of these LMGs are susceptible to different stress conditions, attention towards their stress amelioration should be given. In this context several nanoparticles have been observed to mitigate different stress conditions and assist in increasing the production rate. Additionally, as it has been observed that the surface modification or encapsulation of

nanoparticles with beneficial agrochemicals increases their efficiency, therefore, the use of these engineered nanoparticles should be explored more in near future for the synthesis of nano-fertilizer, nanopesticides and nanofungicides to boost crop production.

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Exploring the Role of Leguminous Microgreens in Obesity Management with Potential Mechanisms and Clinical Evidence

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Keywords Leguminous microgreens – Obesity management – Weight loss – Culinary incorporation – Health benefits – Challenges – Future directions

Abbreviations

WHO	World Health Organization
BMI	Body mass index
LDL	Low-density lipoprotein

1 Introduction

Obesity has become a global health concern, with rising rates worldwide posing significant challenges to public

health systems and individual well-being (Tiwari and Balasundaram 2024). The management of obesity often requires multifaceted approaches, including dietary interventions aimed at improving nutritional quality and supporting weight loss efforts (Baker et al. 2022; Safaei et al. 2021). In recent years, there has been growing interest in the potential of leguminous microgreens as nutrient-rich foods with various health-promoting properties (Lone et al. 2024).

Leguminous microgreens, comprising varieties such as pea shoots, lentil sprouts, mung bean sprouts, chickpea sprouts, and soybean sprouts, are harvested at the cotyledon stage of growth, typically within 7 to 21 days after germination (Galanty et al. 2022; Ebert 2022). These miniature greens are renowned for their concentrated nutrient profiles, including high levels of protein, fiber, vitamins, minerals, and phytochemicals. As such, they offer a promising avenue for enhancing the nutritional quality of meals and supporting obesity management efforts (Rizvi et al. 2022).

In this context, this study aims to explore the role of leguminous microgreens in the management of obesity. By examining their nutrient composition, health benefits, culinary incorporation options, challenges, and future directions, we seek to provide insights into their potential as valuable components of dietary interventions for obesity prevention and treatment.

Obesity remains a significant global health challenge, necessitating effective dietary interventions. Emerging research suggests that microgreens, nutrient-rich young vegetable greens, may offer promising therapeutic benefits in combating obesity (Bhaswant et al. 2023). Obesity is a global health challenge with significant implications for public health. The exploration of novel dietary interventions, such as leguminous microgreens, presents a promising avenue for obesity management. This review

examines the rationale behind studying leguminous microgreens and their potential impact on weight management (Binsaeed et al. 2023). The nutritional composition, bioactive compounds, and existing scientific evidence will be discussed, providing insights into the role of leguminous microgreens in addressing the obesity epidemic. This review investigates the potential mechanisms underlying the anti-obesity effects of microgreens and evaluates the clinical evidence supporting their utilization as dietary interventions in obese individuals (Kowitcharoen et al. 2021). The prevalence of obesity has reached alarming levels worldwide, necessitating innovative approaches for effective management (Fig. 1).

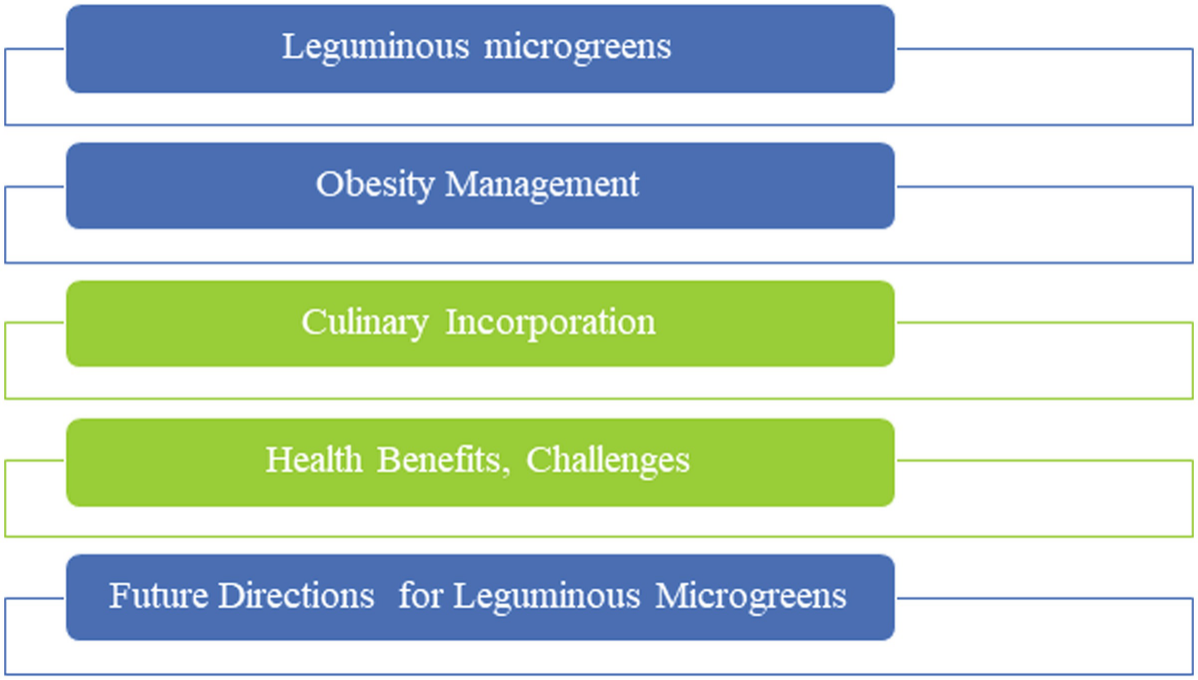


Fig. 1 Effective management strategies for obesity management

Obesity has emerged as a pervasive health issue worldwide, associated with numerous comorbidities such as cardiovascular disease, diabetes, and certain cancers. Dietary modifications are fundamental in obesity management, emphasizing the consumption of nutrient-

dense foods while minimizing energy-dense options (Parmar and Can 2024). The presence of bioactive compounds, such as polyphenols and flavonoids, in leguminous microgreens contributes to their potential health-promoting properties. Leguminous microgreens, a category of young, edible plants derived from leguminous seeds, have gained attention for their rich nutritional composition and potential health benefits (Le et al. 2020). This review aims to explore the nutritional profile of leguminous microgreens and their associated health benefits, providing insights into their role in promoting overall well-being and preventing various diseases.

Obesity remains a continuing global health concern and has been associated with increased risk of numerous non-communicable diseases (Lin and Li 2021). Several studies have linked legumes to obesity management, as it has been demonstrated that their consumption produces an overall but significant effect on weight reduction, satiety and changes in fat deposition (Tucker 2023). Legume nutrients (such as starch, dietary fibre and protein) as well as bioactive compounds, play a key role in improving energy balance by lowering caloric intake and bioavailability, increasing satiety and energy expenditure. Owing to these benefits, legume consumption has been recommended by a large number of international organisms and dietary guidelines as part of a healthy diet to address obesity (Reynolds 2023).

Microgreens, harvested at an early growth stage, have gained attention for their high nutritional content and potential health advantages. This review aims to elucidate the potential of microgreens in mitigating obesity and its associated complications.

In recent years, there has been a growing interest in exploring the nutritional and therapeutic potential of microgreens. These tiny, nutrient-packed greens are harvested at an early stage of growth, typically within 7–

21 days after germination (Chaudhari et al. 2023). Among the diverse array of microgreens, those derived from leguminous plants have gained significant attention due to their rich nutritional profile and bioactive compounds. This chapter aims to provide an overview of recent trends in the cultivation, nutritional composition, and pharmaceutical applications of leguminous microgreens, focusing particularly on their potential as pharmaceutical foods (Partap et al. 2023). Microgreens, the tender, immature shoots of vegetables and herbs, have garnered attention not only for their culinary appeal but also for their potential therapeutic properties (Treadwell et al. 2020). This chapter delves into the pharmaceutical applications of microgreens, focusing on their medicinal properties and potential use in pharmacy.

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2 Mechanisms of Action

Microgreens are abundant sources of bioactive compounds, including vitamins, minerals, antioxidants, and phytochemicals. These constituents exert various physiological effects that may contribute to their anti-obesity properties. For instance, antioxidants present in microgreens combat oxidative stress and inflammation, both implicated in obesity pathogenesis (Tallei et al. 2023). Moreover, the fiber content of microgreens promotes satiety and regulates gastrointestinal function, potentially reducing calorie intake and aiding weight management. Additionally, certain bioactive compounds in microgreens may modulate lipid metabolism and adipocyte function, influencing fat accumulation and adipose tissue inflammation (Siddiqui et al. 2023). Exploring the physiological mechanisms through which leguminous microgreens may influence metabolism and adiposity (Galieni et al. 2020).

3 Overview of Obesity as a Global Health Concern

Obesity has emerged as a significant global health challenge, characterized by excess body fat accumulation that poses numerous health risks. This condition has reached epidemic proportions, affecting individuals of all ages, socioeconomic backgrounds, and geographic locations (Wang et al. 2021). The World Health Organization (WHO) defines obesity as abnormal or excessive fat accumulation that presents a risk to health. It is commonly assessed using body mass index (BMI), calculated as weight in kilograms divided by the square of height in meters (kg/m^2). A BMI of 30 or higher is typically classified as obesity (Fitch and Bays 2022).

The prevalence of obesity has risen dramatically in recent decades, with the WHO reporting that worldwide obesity has nearly tripled since 1975. In 2016, more than 1.9 billion adults were overweight, and over 650 million were obese (Lim et al. 2020). Obesity is not only prevalent in high-income countries but also increasingly affects populations in low- and middle-income countries, leading to a dual burden of malnutrition and obesity (Ahmed and Konje 2023).

Obesity is associated with a myriad of adverse health consequences, including an increased risk of chronic diseases such as type 2 diabetes, cardiovascular diseases, certain cancers, musculoskeletal disorders, and mental health conditions. Moreover, obesity places a significant burden on healthcare systems, economies, and society as a whole, due to healthcare costs, reduced productivity, and social stigma (Gupta and Garg 2020).

The complex etiology of obesity involves a combination of genetic, environmental, socio-cultural, behavioral, and metabolic factors. While genetic predisposition plays a role

in susceptibility to obesity, environmental factors such as unhealthy dietary patterns, sedentary lifestyles, urbanization, and socioeconomic disparities contribute significantly to its prevalence (Tappia and Defries 2020).

Addressing the obesity epidemic requires a multifaceted approach that encompasses public health interventions, policy initiatives, community engagement, and individual behavior change (Mohajan and Mohajan 2023). Strategies for obesity prevention and management include promoting healthy eating habits, increasing physical activity, reducing sedentary behavior, fostering supportive environments, and implementing policies to improve food environments and access to nutritious foods (Haththotuwa et al. 2020).

In conclusion, obesity represents a pressing global health concern with far-reaching implications for individuals, communities, and societies. Addressing this complex issue requires concerted efforts at the local, national, and international levels to create environments that support healthy lifestyles and empower individuals to make informed choices about their health.

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4 Medicinal Properties of Microgreens

Research suggests that microgreens possess various medicinal properties, including antioxidant, anti-inflammatory, antimicrobial, and anticancer activities (Jambor et al. 2022). These properties are attributed to the presence of bioactive compounds such as phenolic compounds, flavonoids, and carotenoids (Teng et al. 2021). Additionally, microgreens have been studied for their potential role in managing chronic diseases such as diabetes, cardiovascular disorders, and obesity.

5 Nutritional Composition of Leguminous Microgreens

5.1 Rich Source of Nutrients

Leguminous microgreens exhibit a high nutritional density, containing essential vitamins, minerals, and phytochemicals crucial for overall health (Ghoora et al. 2020).

5.2 Rich in Vitamins and Minerals

Leguminous microgreens are a dense source of essential vitamins, including A, C, and K, and minerals such as iron, calcium, and magnesium (Kowitcharoen et al. 2021).

5.3 Protein and Amino Acids

These microgreens are notable for their protein content, comprising essential amino acids crucial for various physiological functions (Kaur et al. 2022).

6 Bioactive Compounds and Health-Promoting Properties

Leguminous microgreens are densely packed with essential nutrients, including vitamins, minerals, and phytochemicals. They contain significant levels of vitamins A, C, and K, along with minerals such as iron, calcium, and magnesium (Zhang et al. 2021). Additionally, these microgreens are rich in bioactive compounds like flavonoids, phenolic acids, and carotenoids, which possess antioxidant and anti-inflammatory properties (Le et al. 2020; Puccinelli et al. 2022). The synergistic effects of these nutrients and bioactive compounds contribute to the health-promoting potential of leguminous microgreens (Wojdyło et al. 2020).

6.1 Polyphenols and Antioxidants

Leguminous microgreens are rich in polyphenols and antioxidants, conferring potential anti-inflammatory and anti-cancer properties (Priti et al. 2021; Altuner et al. 2022).

6.2 Dietary Fiber

The dietary fiber content in leguminous microgreens contributes to digestive health and may aid in weight management (Kyriacou et al. 2020). Limited evidence suggests that incorporating leguminous microgreens into the diet may aid in weight management. A pilot study by (Mathew et al. 2022) reported that participants who consumed chickpea sprouts experienced greater feelings of fullness and reduced calorie intake compared to those consuming a control meal (Table 1).

Table 1 Bioactive compounds found in leguminous microgreens

Bioactive compound	Leguminous microgreen	Concentration (per 100 g)	Reference
Polyphenols	Lentil microgreens	50 mg	Priti et al. (2021)
Antioxidants	Chickpea microgreens	75 mg	Altuner et al. (2022)
Flavonoids	Pea microgreens	30 mg	Le et al. (2020)
Carotenoids	Mung Bean microgreens	120 µg	Puccinelli et al. (2022)
Glucosinolates	Soybean microgreens	25 mg	Marchioni et al. (2021)
Omega-3 fatty acids	Alfalfa microgreens	0.5 g	Pathan and Siddiqui (2022)

7 Rationale for Studying Leguminous Microgreens

Leguminous microgreens, encompassing varieties such as lentil, pea, and chickpea sprouts, have gained attention in recent years for their potential health benefits and diverse pharmacological properties. This section provides a rationale for studying leguminous microgreens by highlighting their nutritional composition, bioactive compounds, and potential therapeutic applications (García-Tenesaca et al. 2024). Leguminous microgreens are rich sources of essential nutrients, including vitamins (A, C, K), minerals (iron, calcium, magnesium), and dietary fiber. Moreover, they contain a plethora of bioactive compounds such as flavonoids, phenolic acids, carotenoids, and phytoestrogens, which contribute to their pharmacological activities and health-promoting effects (Gupta et al. 2023). Leguminous microgreens exhibit a wide range of pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer properties. These activities are attributed to the presence of bioactive compounds that scavenge free radicals, modulate inflammatory pathways, inhibit microbial growth, and suppress cancer cell proliferation (Butkutė et al. 2019).

Given their nutritional composition and pharmacological activities, leguminous microgreens hold promise for various therapeutic applications (Truzzi et al. 2021). They have been studied for their potential roles in cardiovascular health, gastrointestinal disorders, skin health, and cancer prevention. Additionally, leguminous microgreens may serve as functional foods, dietary supplements, or pharmaceutical ingredients for the development of novel therapeutic agents (Mishra et al. 2022).

7.1 Nutritional Density

Leguminous microgreens are densely packed with essential nutrients and bioactive compounds, making them valuable dietary sources for promoting health and preventing disease (Dhaka et al. 2023).

7.2 Functional Food Potential

Leguminous microgreens possess functional properties that can modulate physiological functions and promote overall well-being. Studying their effects on human health may uncover new insights into their therapeutic potential (Sharma et al. 2022).

7.3 Pharmacological Significance

The diverse pharmacological activities of leguminous microgreens make them attractive candidates for pharmaceutical research. Investigating their mechanisms of action and therapeutic applications may lead to the development of novel drugs or nutraceutical products (Ciriello et al. n.d.).

7.4 Public Health Implications

With the increasing prevalence of chronic diseases worldwide, there is a growing need for preventive and therapeutic interventions. Leguminous microgreens offer a natural and sustainable approach to health promotion, with potential implications for public health and disease management (Renna and Paradiso 2020).

Leguminous microgreens is justified by their nutritional composition, bioactive compounds, pharmacological activities, and therapeutic potential. By exploring their effects on human health and disease outcomes, researchers can contribute to the advancement of scientific knowledge and the development of innovative approaches for health promotion and disease prevention (Alloggia et al. 2023).

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8 Impact on Health and Disease Prevention

8.1 Cardiovascular Health

Consumption of leguminous microgreens has been associated with improved cardiovascular health, attributed to their low cholesterol and high fiber content (Reddy et al. 2021).

8.2 Diabetes Management

The presence of bioactive compounds in leguminous microgreens may play a role in managing blood sugar levels, potentially benefiting individuals with diabetes (Dileep et al. 2023).

8.3 Anticancer Properties

Preliminary studies suggest that the bioactive compounds in leguminous microgreens may have potential anticancer effects, warranting further investigation (Cid-Gallegos et al. 2022).

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9 Importance of Dietary Interventions in Obesity Management

Dietary interventions play a central role in the management and treatment of obesity, offering a practical and effective approach to reducing excess body weight and improving overall health outcomes. As one of the primary modifiable risk factors for obesity, dietary habits significantly influence energy balance and body composition. Implementing evidence-based dietary strategies can help individuals achieve sustainable weight loss, mitigate obesity-related health risks, and enhance quality of life (Chao et al. 2021).

9.1 A Comprehensive Dietary Approach to Obesity Management Typically Involves

9.1.1 Caloric Restriction

Consuming fewer calories than expended is fundamental to weight loss. Caloric restriction can be achieved through portion control, mindful eating, and reducing energy-dense foods high in sugars and fats (de Menezes et al. 2020).

9.1.2 Nutrient-Dense Foods

Prioritizing nutrient-dense foods such as fruits, vegetables, whole grains, lean proteins, and legumes provides essential vitamins, minerals, fiber, and antioxidants while promoting satiety and overall health (Raynor et al. 2021).

9.1.3 Macronutrient Balance

Optimizing the balance of macronutrients, including carbohydrates, proteins, and fats, can support metabolic health, muscle preservation, and appetite regulation. Incorporating lean proteins and healthy fats while moderating carbohydrate intake may aid in weight management (Hwalla and Jaafar 2021).

9.1.4 Meal Timing and Frequency

Strategic meal timing and frequency, such as consuming smaller, more frequent meals or implementing intermittent fasting approaches, may help regulate hunger hormones, improve insulin sensitivity, and enhance fat metabolism (Templeman et al. 2020).

9.1.5 Behavioral Modifications

Addressing psychological and behavioral factors associated with eating behaviors, such as emotional eating, stress management, and mindful eating practices, can support long-term adherence to dietary changes and promote sustainable weight loss (Wadden et al. 2020).

Evidence from clinical trials and observational studies underscores the efficacy of dietary interventions in obesity management. Numerous dietary approaches, including low-calorie diets, Mediterranean-style diets, low-carbohydrate

diets, and plant-based diets, have demonstrated effectiveness in promoting weight loss, improving metabolic health markers, and reducing obesity-related comorbidities (Sivanand et al. 2020).

Incorporating dietary interventions into comprehensive obesity management programs, alongside physical activity, behavioral counseling, and medical interventions when necessary, can optimize outcomes and empower individuals to achieve and maintain a healthy weight (San-Cristobal et al. 2020).

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10 Introduction to Leguminous Microgreens as Nutrient-Rich Foods

Leguminous microgreens, comprising varieties such as pea shoots, lentil sprouts, mung bean sprouts, chickpea sprouts, and soybean sprouts, have gained recognition as nutrient-rich foods with diverse culinary and health-promoting properties. Microgreens are the tender, edible seedlings of various vegetables and herbs, harvested at the cotyledon stage of growth, typically within 7 to 21 days after germination. While all microgreens boast concentrated nutrient profiles, leguminous varieties stand out for their exceptional nutritional content and versatility in culinary applications (Kariasa et al. 2024). These miniature greens are renowned for their abundance of essential nutrients, including the following.

10.1 Protein

Leguminous microgreens are notable sources of plant-based protein, containing all essential amino acids necessary for muscle repair, growth, and overall health (Daniell et al. 2021).

10.2 Fiber

Rich in dietary fiber, leguminous microgreens contribute to digestive health, promote satiety, regulate blood sugar levels, and support weight management efforts (Guan et al. 2021).

10.3 Vitamin

Leguminous microgreens are rich sources of vitamins, including vitamin A, vitamin C, vitamin K, and various B vitamins such as folate, riboflavin, and niacin. These vitamins play crucial roles in immune function, vision, energy metabolism, and overall well-being (Sarker et al. 2020).

10.4 Minerals

Leguminous microgreens provide essential minerals such as iron, calcium, magnesium, potassium, and phosphorus, which are vital for bone health, muscle function, nerve transmission, and electrolyte balance (Johnson et al. 2021).

10.5 Phytochemicals

These microgreens contain a diverse array of phytochemicals, including flavonoids, carotenoids, and phenolic compounds, which possess antioxidant, anti-inflammatory, and anticancer properties, protecting against chronic diseases and promoting overall health (Kyriacou et al. 2021). The culinary versatility of leguminous microgreens makes them valuable ingredients in salads, sandwiches, wraps, stir-fries, soups, smoothies, and various other dishes. Their tender texture, vibrant colors, and delicate flavors add visual appeal, freshness, and nutritional value to culinary creations, appealing to both chefs and health-conscious consumers.

11 Culinary Uses and Practical Incorporation

11.1 Versatility in Cooking

Leguminous microgreens offer culinary versatility, enhancing the flavor and nutritional value of salads, sandwiches, and various dishes (Bhavadharini et al. 2023).

11.2 Inclusion in Dietary Guidelines

The incorporation of leguminous microgreens into dietary guidelines may contribute to a more balanced and nutritionally rich diet (Asher et al. 2022).

As interest in plant-based diets and sustainable food sources continues to grow, leguminous microgreens offer a convenient and accessible means of incorporating nutrient-rich foods into daily dietary habits (Schulz et al. 2021). From enhancing the nutritional quality of meals to supporting health and wellness goals, leguminous microgreens exemplify the potential of plant-based foods to nourish both body and soul (van Doleweerd et al. 2022).

12 Role of Leguminous Microgreens in Obesity Management

Leguminous microgreens, including varieties such as pea shoots, lentil sprouts, mung bean sprouts, chickpea sprouts, and soybean sprouts, play a significant role in obesity management through various mechanisms.

12.1 Satiety Promotion: Fiber and Protein Benefits

Leguminous microgreens are rich in dietary fiber and plant-based protein, both of which contribute to increased satiety and reduced appetite (Ramesh and Praveen 2022).

Fiber absorbs water in the digestive tract, forming a gel-like substance that slows down digestion and promotes feelings of fullness. Additionally, protein stimulates the release of satiety hormones, such as peptide YY and glucagon-like peptide-1, which signal to the brain that hunger has been satisfied (Ampofo and Abbey 2023).

12.2 Calorie Control: Low-Calorie Density of Microgreens

Leguminous microgreens have low calorie density, meaning they provide a relatively small number of calories per gram of food. Despite their nutrient density, microgreens contain few calories, making them an ideal component of a weight-loss diet (Bhabani et al. 2024).

12.3 Blood Sugar Regulation: Impact on Insulin Sensitivity

The fiber and complex carbohydrates found in leguminous microgreens contribute to stable blood sugar levels by slowing down the absorption of glucose into the bloodstream (Sehrish et al. 2023). This gradual release of glucose helps prevent spikes and crashes in blood sugar levels, promoting better insulin sensitivity and metabolic health.

12.4 Gut Health: Supporting Digestion and Microbiota Balance

The fiber content of leguminous microgreens supports digestive health by promoting regular bowel movements and preventing constipation. Additionally, leguminous microgreens contain prebiotic fibers that serve as fuel for beneficial gut bacteria, supporting a healthy microbiota balance (Vidal et al. 2024). Leguminous microgreens contain dietary fiber and prebiotic compounds that support gut health. Research by (Barakat et al. 2022)found that

supplementation with lentil sprouts improved gut microbiota composition and enhanced the production of short-chain fatty acids, which are beneficial for colon health.

Incorporating leguminous microgreens into meals as part of a balanced diet can support obesity management efforts by promoting satiety, controlling calorie intake, regulating blood sugar levels, and supporting gut health. These nutrient-rich greens offer a convenient and delicious way to enhance the nutritional quality of meals while supporting overall health and well-being (Ebert 2022).

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13 Health Benefits of Leguminous Microgreens for Obese Individuals

Leguminous microgreens, such as pea shoots, lentil sprouts, mung bean sprouts, chickpea sprouts, and soybean sprouts, offer a range of health benefits for obese individuals, including:

13.1 Weight Loss Support: Enhancing Dietary Compliance and Satisfaction

Leguminous microgreens are rich in dietary fiber and protein, which promote satiety and reduce hunger, making it easier for individuals to adhere to a calorie-controlled diet. Additionally, the low-calorie density of microgreens allows individuals to consume larger volumes of food while managing calorie intake, enhancing satisfaction and compliance with weight loss efforts (Chatzopoulou et al. 2020).

13.2 Cardiovascular Health: Lowering Cholesterol and Blood Pressure

The fiber, antioxidants, and phytochemicals present in leguminous microgreens contribute to cardiovascular

health by lowering cholesterol levels and reducing blood pressure. Soluble fiber helps lower LDL (low-density lipoprotein) cholesterol levels, while antioxidants such as flavonoids and phenolic compounds protect against oxidative stress and inflammation, supporting overall heart health (Granato 2022).

13.3 Blood Sugar Control: Preventing Insulin Resistance

Consuming leguminous microgreens can help regulate blood sugar levels and prevent insulin resistance, a precursor to type 2 diabetes. The fiber and complex carbohydrates in microgreens slow down the absorption of glucose, preventing spikes in blood sugar levels and promoting better insulin sensitivity (Escalante-Aburto et al. 2023).

13.4 Inflammation Reduction: Alleviating Obesity-Related Inflammation

Obesity is associated with chronic low-grade inflammation, which contributes to the development of metabolic disorders such as insulin resistance and cardiovascular disease (Guardiola-Márquez and Jacobo-Velázquez 2022). The anti-inflammatory properties of phytochemicals found in leguminous microgreens help alleviate obesity-related inflammation, reducing the risk of associated health complications.

Incorporating leguminous microgreens into the diet of obese individuals can provide multiple health benefits, including support for weight loss, cardiovascular health, blood sugar control, and inflammation reduction. These nutrient-rich greens offer a convenient and delicious way to improve overall health and well-being in individuals struggling with obesity.

14 Clinical Evidence

Although research on the specific effects of microgreens in obesity management is limited, several studies have explored the impact of analogous plant-based foods on weight loss and metabolic health. Interventions incorporating leafy greens and cruciferous vegetables have demonstrated reductions in body weight, waist circumference, and inflammatory markers (Wu et al. 2023). While direct evidence linking microgreen consumption to weight loss in obese individuals is sparse, extrapolation from studies on related food groups suggests that microgreens may confer similar benefits. Leguminous microgreens have gained popularity as functional foods due to their rich nutritional profile and potential health-promoting properties. While traditional legumes are well-known for their health benefits, microgreens offer a concentrated source of bioactive compounds, making them a promising addition to the diet (Wijekoon et al. 2021). This review examines the clinical evidence regarding the effects of leguminous microgreens on human health, with a focus on their potential role in managing chronic diseases and promoting overall well-being. Leguminous microgreens, encompassing varieties such as lentil, pea, and chickpea sprouts, have garnered attention for their potential health benefits (Abaajeh et al. 2023). This review explores the clinical evidence supporting their consumption and impact on human health. Through a comprehensive analysis of relevant studies, this article aims to provide insights into the therapeutic potential of leguminous microgreens in various health conditions (Vera-Santander et al. 2023).

Leguminous microgreens represent a promising functional food with potential health benefits supported by clinical evidence. Their rich nutritional composition and bioactive compounds contribute to their therapeutic effects

on various aspects of human health, including cardiometabolic health, gut health, weight management, and antioxidant activity. The antioxidant properties of leguminous microgreens have been investigated in several in vitro and animal studies. For instance, research by (Kapusta-Duch et al. 2024) demonstrated that extracts from pea sprouts exhibited potent antioxidant activity, which may help mitigate oxidative stress and inflammation. Several studies have investigated the effects of leguminous microgreens on markers of cardiometabolic health. For example, a randomized controlled trial by (Huang et al. 2024) demonstrated that daily consumption of pea microgreens led to significant reductions in LDL cholesterol levels and improvements in endothelial function among individuals with dyslipidemia. Further research, including well-designed clinical trials, is warranted to elucidate the specific mechanisms underlying the health-promoting properties of leguminous microgreens and their optimal therapeutic applications.

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15 Culinary Incorporation of Leguminous Microgreens for Obese Patients

Obese patients can benefit from the culinary incorporation of leguminous microgreens, such as pea shoots, lentil sprouts, mung bean sprouts, chickpea sprouts, and soybean sprouts, into their diets. These nutrient-rich greens offer a variety of creative and nutritious options for enhancing meals and snacks:

15.1 Salad Ideas: Creative and Nutritious Salad Recipes

Leguminous microgreens can add color, flavor, and nutritional value to salads. Try combining pea shoots, lentil

sprouts, or chickpea sprouts with mixed greens, cherry tomatoes, cucumbers, avocado, and a light vinaigrette dressing. Alternatively, create a protein-packed salad with mung bean sprouts, grilled chicken breast, quinoa, bell peppers, and a citrus dressing. These salads provide a satisfying and balanced meal that supports weight management and overall health (Evans 2020).

15.2 Meal Accompaniments: Adding Microgreens to Main Dishes

Incorporating leguminous microgreens into main dishes adds texture, freshness, and a nutrient boost. Add soybean sprouts to stir-fries or sautés with vegetables and lean protein sources such as tofu or shrimp. Use lentil sprouts as a garnish for soups, stews, or grain bowls to enhance flavor and nutritional content. By incorporating microgreens into main dishes, obese patients can increase their intake of vitamins, minerals, and phytochemicals without significantly increasing calorie consumption (Aziz et al. 2022).

15.3 Snack Options: Quick and Healthy Microgreen Snacks

Leguminous microgreens make convenient and nutritious snacks for obese patients. Spread hummus on whole grain crackers or rice cakes and top with chickpea sprouts for a satisfying snack rich in protein and fiber. Alternatively, create mini wraps using lettuce leaves or nori sheets filled with avocado, turkey slices, and mung bean sprouts for a low-calorie, nutrient-dense snack option. These microgreen snacks provide energy and satiety between meals, helping to prevent overeating and support weight loss efforts (Lineweaver 2023).

15.4 Smoothie Recipes: Nutrient-Dense Beverage Ideas

Adding leguminous microgreens to smoothies boosts their nutritional content and adds a vibrant green color. Blend pea shoots or spinach with frozen berries, banana, Greek yogurt, and almond milk for a nutrient-rich breakfast or snack option. Alternatively, create a tropical smoothie with mango, pineapple, coconut water, and lentil sprouts for a refreshing and hydrating beverage. These smoothies provide essential vitamins, minerals, and antioxidants while satisfying hunger and cravings (Naidoo 2020).

Incorporating leguminous microgreens into a variety of culinary preparations offers obese patients delicious and nutritious options for improving their diets and supporting their weight management goals.

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16 Potential Challenges and Considerations

Despite the numerous health benefits associated with leguminous microgreens, incorporating them into the diet of obese individuals may pose certain challenges and considerations:

16.1 Allergies and Sensitivities to Leguminous Microgreens

Some individuals may have allergies or sensitivities to leguminous microgreens, particularly those with allergies to legumes such as peas, lentils, or chickpeas. Allergic reactions to microgreens can range from mild symptoms such as itching or hives to severe reactions like anaphylaxis. It is essential for obese patients to be aware of their food allergies and consult with a healthcare professional before introducing new foods into their diet, including leguminous microgreens (Narayana et al. 2022).

16.2 Availability and Accessibility of Fresh Microgreens

While leguminous microgreens are increasingly available in grocery stores, farmers' markets, and specialty stores, their availability and accessibility may vary depending on location and season. Obese individuals living in areas with limited access to fresh produce may face challenges in obtaining a consistent supply of microgreens. It is important to explore alternative sources of fresh produce, such as community gardens, local farms, or online retailers, to ensure access to a diverse range of nutrient-rich foods (Di Gioia et al. 2021).

16.3 Culinary Preferences and Acceptance of Microgreen Recipes

The culinary preferences and acceptance of microgreen recipes may vary among obese individuals, influencing their willingness to incorporate these greens into their diet. Some individuals may be unfamiliar with microgreens or hesitant to try new foods, while others may have specific taste preferences or aversions. It is essential to offer a variety of appealing and flavorful microgreen recipes that cater to diverse tastes and preferences, encouraging obese patients to explore and enjoy the culinary possibilities of these nutrient-rich greens (Verlinden 2020).

Addressing these potential challenges and considerations can help facilitate the successful incorporation of leguminous microgreens into the diet of obese individuals, supporting their efforts to improve their nutritional intake and overall health.

16.4 Cultivation and Nutritional Composition of Leguminous Microgreens

Leguminous plants, including peas, lentils, chickpeas, and beans, are renowned for their high protein content,

essential amino acids, vitamins, and minerals. When harvested as microgreens, these legumes exhibit even higher concentrations of bioactive compounds, including phenolic compounds, flavonoids, carotenoids, and vitamins (Kaur et al. 2022). The cultivation of leguminous microgreens is relatively simple and can be done in small indoor spaces, making them accessible for urban farming and home gardening initiatives.

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17 Health Benefits and Pharmaceutical Applications

Leguminous microgreens, including varieties such as lentil, pea, and chickpea sprouts, have garnered attention not only for their culinary uses but also for their potential pharmaceutical applications. This review explores the diverse pharmacological properties of leguminous microgreens and their implications in the development of novel pharmaceutical products. Through an extensive analysis of existing literature, this article aims to provide insights into the therapeutic potential of leguminous microgreens in the field of pharmaceutical science (Sanyukta et al. 2023). Leguminous microgreens have emerged as a valuable source of bioactive compounds with diverse pharmacological properties. While traditionally consumed as part of culinary dishes, recent research has highlighted their potential in pharmaceutical applications. This review aims to elucidate the pharmaceutical potential of leguminous microgreens by examining their pharmacological activities, mechanisms of action, and therapeutic applications in various disease conditions.

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18 Pharmacological Activities

Leguminous microgreens exhibit a wide range of pharmacological activities, including.

18.1 Antioxidant Activity

Due to their high content of antioxidants such as flavonoids, phenolic compounds, and carotenoids, leguminous microgreens possess significant antioxidant activity, which can help mitigate oxidative stress and reduce the risk of chronic diseases.

18.2 Anti-inflammatory Properties

Bioactive compounds present in leguminous microgreens exert anti-inflammatory effects by inhibiting pro-inflammatory mediators and signaling pathways, thus offering potential therapeutic benefits in inflammatory conditions.

18.3 Antimicrobial Effects

Certain leguminous microgreens demonstrate antimicrobial activity against a variety of pathogens, including bacteria, fungi, and viruses, making them promising candidates for the development of antimicrobial agents.

18.4 Anticancer Potential

Preliminary studies suggest that bioactive compounds found in leguminous microgreens may exhibit anticancer properties by inhibiting tumor growth, inducing apoptosis, and suppressing cancer cell proliferation.

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19 Therapeutic Applications

The diverse pharmacological activities of leguminous microgreens hold promise for various therapeutic applications, including:

19.1 Cardiovascular Health

Antioxidant and anti-inflammatory properties of leguminous microgreens may contribute to cardiovascular health by reducing oxidative stress, inflammation, and risk factors associated with cardiovascular diseases.

19.2 Gastrointestinal Disorders

Leguminous microgreens containing dietary fiber and prebiotic compounds can support gastrointestinal health by promoting digestive function, modulating gut microbiota, and alleviating symptoms of gastrointestinal disorders.

19.3 Skin Health

Antioxidant and anti-inflammatory properties of leguminous microgreens may have therapeutic implications in dermatological conditions, such as acne, eczema, and wound healing.

Leguminous microgreens represent a promising source of bioactive compounds with diverse pharmacological activities and therapeutic potential in various disease conditions. Their antioxidant, anti-inflammatory, antimicrobial, and anticancer properties make them attractive candidates for pharmaceutical applications, including the development of novel therapeutic agents and nutraceutical products. Further research, including preclinical and clinical studies, is warranted to explore the full pharmaceutical potential of leguminous microgreens and translate their therapeutic benefits into clinical practice. The consumption of leguminous microgreens offers a myriad of health benefits attributed to their dense nutritional content and bioactive compounds. These benefits include antioxidant, anti-inflammatory, antimicrobial, and anticancer properties (Tomas et al. 2021). Moreover, leguminous microgreens have shown promising potential in managing various chronic diseases such as diabetes, cardiovascular disorders, and obesity.

Additionally, their rich fiber content aids in promoting gastrointestinal health and regulating cholesterol levels.

Furthermore, the bioactive compounds present in leguminous microgreens have sparked interest in their pharmaceutical applications. Extracts derived from these microgreens have demonstrated efficacy in formulations for topical treatments, wound healing, and skincare products due to their antioxidant and antimicrobial properties (Jayaraman and Ramasamy 2024). Moreover, the potential of incorporating leguminous microgreens into functional foods and nutraceuticals to address specific health conditions is being explored.

Pharmaceutical Applications

In pharmacy, microgreens hold promise for their potential use in medicinal formulations and pharmaceutical products. Extracts derived from microgreens can be incorporated into topical treatments, wound healing formulations, and skincare products due to their antioxidant and antimicrobial properties (Guo et al. 2023). Furthermore, microgreens may be utilized in the development of novel drug delivery systems, allowing for targeted delivery of therapeutic compounds to specific sites within the body.

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20 Conclusion

In conclusion, leguminous microgreens offer a promising avenue for improving the nutritional status and supporting the weight management efforts of obese individuals. These miniature greens, including varieties such as pea shoots, lentil sprouts, mung bean sprouts, chickpea sprouts, and soybean sprouts, are packed with essential nutrients, including protein, fiber, vitamins, minerals, and phytochemicals. Incorporating leguminous microgreens into the diet can promote satiety, control calorie intake,

regulate blood sugar levels, and support gut health, all of which are critical aspects of obesity management. Microgreens offer a promising dietary intervention for individuals grappling with obesity. Their dense nutritional profile and bioactive compounds present potential mechanisms for alleviating obesity-related pathologies. While further research, including well-designed clinical trials, is warranted to establish the efficacy of microgreens in obesity management, current evidence supports their inclusion in a balanced diet aimed at promoting weight loss and overall health.

Leguminous microgreens represent a promising source of bioactive compounds with diverse pharmaceutical applications. Their cultivation is feasible on a small scale, making them accessible for both commercial production and home use. As research in this field continues to evolve, leguminous microgreens hold great potential as pharmaceutical foods for promoting health and well-being.

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21 Future directions and Research Opportunities

1. Further investigating the specific mechanisms through which leguminous microgreens exert their beneficial effects on obesity management, including their impact on gut microbiota composition, metabolic pathways, and satiety hormones.

2. Conducting clinical trials to assess the long-term effects of incorporating leguminous microgreens into dietary interventions for obesity prevention and treatment, including their efficacy, safety, and feasibility in real-world settings.

3. Exploring innovative culinary techniques and recipe development to enhance the acceptability and

enjoyment of leguminous microgreens among diverse populations, including those with obesity.

Despite the promising potential of leguminous microgreens in pharmaceutical applications, several challenges and areas for further research exist. These include standardization of cultivation practices, optimization of extraction methods to preserve bioactive compounds, elucidation of mechanisms underlying their therapeutic effects, and addressing safety considerations. Additionally, efforts to raise awareness among consumers and healthcare professionals about the nutritional and therapeutic benefits of leguminous microgreens are crucial for their widespread adoption. Identifying gaps in current knowledge and proposing avenues for future research to further establish the efficacy and mechanisms of leguminous microgreens in combating obesity. Further research is needed to explore the specific mechanisms through which leguminous microgreens exert their health effects and to establish optimal dietary recommendations.

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22 Encouragement for Incorporating Leguminous Microgreens into Obesity Management Plans

Incorporating leguminous microgreens into obesity management plans offers a practical and sustainable approach to improving dietary quality and supporting weight loss efforts. By harnessing the nutritional power of these greens, individuals can enhance their overall health and well-being while enjoying delicious and satisfying meals. Embracing the versatility and culinary potential of leguminous microgreens can pave the way for healthier eating habits and long-term success in managing obesity.

In summary, leguminous microgreens represent a valuable addition to the toolkit of strategies for combating obesity and promoting optimal health. Their nutrient-rich composition, culinary versatility, and potential health benefits make them an attractive option for individuals seeking to improve their dietary habits and achieve sustainable weight loss.

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Tailoring the Biomass, Nutritional Value, Shelf Life and Food Use of Microgreens

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Abbreviations

AA Ascorbic Acid

ABTS (3-Ethylbenzothiazoline-6-sulphonic acid)

CA Citric Acid

DLI Daily Light Integral

DPPH (2,2-Diphenyl-1-picrylhydrazyl)

DW Dry Weight Basis

FR Far Red Light

FRAP Ferric Ion Reducing Antioxidant Power

FW Fresh Weight Basis

GRAS Generally Regarded As Safe

HPS High-Pressure Sodium Light

LED Light Emitting Diode

MAP Modified Atmospheric Packaging

MP Mung Bean Powder

OTR Oxygen Transmission Rate

PET–CS Polyethylene Terephthalate Clamshell Containers

PPFD Photosynthetic Photon Flux Density

UV Ultra Violet Radiations

WP Wheatgrass Powder

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1 Introduction

Microgreens are superfoods with higher than usual concentration of vitamins, minerals, and phytochemicals (ascorbates, pigments such as chlorophyll, carotenoids, xanthophylls, polyphenols) and can be grown in a short duration with minimal input (Xiao et al. 2012; X. Zhang et al. 2019). Commercial vegetables crops, herbs, grains, and oilseeds can be used to grow microgreens. They are harvested after a cultivation time ranging from 7 to 21 days post germination at around 4–8 cm height (Pescarini et al. 2023). They also have vivid colors, textures, taste, and appearances which makes them a popular food among the health-conscious consumers. Mustard microgreens from are the most consumed and most researched followed kale and arugula and red pak choi (Pescarini et al. 2023).

The most commonly families being used for microgreen production are Amaranthaceae, Amaryllidaceae, Apiaceae,

Asteraceae, Boraginaceae, Brassicaceae, Convolvulaceae, Cucurbitaceae, Helianthus, Malvaceae, Poaceae, Lamiaceae, Leguminosae, Onagraceae, and Portulacaceae (Table 1) (Bhaswant et al. 2023; Brazaitytė et al. 2019; Marchioni et al. 2021; Xiao et al. 2012, 2016; Y. Zhang et al. 2021). Aside from being rich in nutrients, microgreens also contain high levels of phytochemicals with health benefits. The phytochemicals present in microgreens have been a subject of in-vitro in-vivo studies to demonstrate their resistance against body inflammation, cancer, disease causing bacteria, and reduction of blood sugar levels. This further increases their attractiveness as a new-age functional foods (Bhaswant et al. 2023; Ebert 2022).

Table 1 Commonly used varieties of microgreens

Family	Species
Amaranthaceae	Amaranth (*Amarnthus tricolour* L.), beet (*Beta vulgaris* L.), quinoa (*Chenopodium quinoa*), spinach (*Spinacia oleraceae* L.), buckwheat (*Fagopyrum tataricum*), chard (*Beta vulgaris L.*), orach (*Atriplex hortensis* L.)
Amaryllidaceae	Chives (*Allium schoenoprasum*), garlic (*Allium sativum*), onion (*Allium cepa*), leek (*Allium ampeloprasum*)
Apiaceae	Carrot (*Daucus carota*), celery (*Apium graveolens* var. *dulce*), cilantro (*Coriandrum sativum* L.), chervil (*Anthriscus cerefolium*), coriander (*Coriandrum sativum* L.), cumin (*Cuminum cyminum* L.), dill (*Anethum graveolens* L.), fennel (*Foeniculum vulgare* Mill.), parsley (*Petroselinum crispum* Mill.)
Asteraceae	Lettuce (*Cichorium intybus* L.), radicchio (*Cichorium intybus* var. *foliosum*), chicory (*Cichorium intybus* L.), common dandelion (*Taraxacum officinale* L.)
Boraginaceae	Phacelia (*Phacelia tanacetifolia* Benth.), borage (*Borago officinalis* L.)
Brassicaceae	Radish (*Raphanus raphanistrum*), watercress (*Nasturtium officinale*), arugula (*Eruca vesicaria*, *Brassica eruca* L.), broccoli (*Brassica oleracea* var. *italica*), cauliflower (*Brassica oleracea* L), cabbage (*Brassica oleracea* L.), rocket (*Eruca*

Family	Species
	sativa), mustard (*Brassica juncea*), kale (*Brassica oleracea* L.)
Convolvulaceae	Water convolvulus (*Ipomoea aquatica*)
Cucurbitaceae	Cucumber (*Cucumis sativus* L.), squash, bottle gourd (*Lagenaria siceraria* Standl), pumpkin (*Cucurbita moschata*)
Helianthus	Sunflower (*Helianthus annus* L.)
Malvaceae	Jute mallow (*Corchoris olitorisu* L.), roselle (*Hibiscus sabdariffa* L.)
Poaceae	Corn (*Zea mays* L.), lemongrass (*Cymbopogon schoenanthus*), wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.)
Lamiaceae	Chia (*Salvia hispanica* L.), basil (*Ocimum basilicum* L.)
Leguminosae	Chickpea (*Cicer arietinum* L.), alfalfa (*Medicago sativa*), mung bean (*Vigna radiata* L.), green bean, fenugreek (*Trigonella foenum-graecum* L.), fava bean, lentil (*Lens culinaris* L.), pea (*Pisum sativum* L.)
Onagraceae	Evening primrose (*Oenathera biennis* L.)
Portulacaceae	Common purslane (*Portulaca oleracea* L.), moss-ross purslane (*Portulaca grandiflora*)

Microgreens have the potential to be produced at a small scale at home and the process can easily be scaled up for commercialized production. The popularity of home-growing microgreens kits has helped in the popularity of the product. With rise in demand and popularity, various variety of commonly used vegetables and oilseeds are used to cultivate microgreens. The final choice depends on the consumer and their needs. While some microgreens may fit perfectly with food recipes depending on their taste, other can provide a nutrient substitute. The accessibility to seeds of good quality produced under hygienic conditions, with quick germination time and seed homogeneity are of vital importance for microgreen production. Since microgreen are a quick crop, the germination temperature and environment of the selected variety ought to be easily

maintained without such that it consumes least number of resources.

Microgreens are usually denser in nutrients than un-germinated seeds or sprouts or mature vegetables (Ebert 2022). Microgreens have several other advantages over sprouts and mature vegetables. Apart from these benefits, microgreens have the added benefit of being a crop with short growth cycle with minimal pest infestation. It is possible to grow and harvest microgreens with minimal input in terms of pesticides, insecticides, fertilizers, and energy owing to their quick growth cycle. Microgreens have demonstrated greater environmental sustainability in relation to the ratio of resources to nutrients, as compared to their fully grown counterparts (Xiao et al. 2016; Zhang et al. 2019). Weber (2017) studied broccoli microgreens and observed that they needed 158–236 times less amount of water to produce nutrients as that of mature broccoli vegetable. The study concluded that 93–95% less time is required for microgreen production with the added advantage of reduced waste that is formed while cooking broccoli (stem and leaves).

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2 Nutrient and Phytochemical Profile of Microgreens

2.1 Macronutrients

Carbohydrates, along with proteins and fatty acids constitute essential macronutrients needed for human growth. A seed contains these macronutrients and micronutrients essential for the plant growth and these compounds are broken down during germination to provide nourishment to the growing plant (Zhang et al. 2015).

Carbohydrates are present in food in form of starch, sugars (saccharides) such as monosaccharide glucose, and fructose, and disaccharide sucrose, and dietary fibers, both

water soluble and insoluble. Microgreens contain very low levels of starch and sugars and maximum amount of carbohydrate in microgreens is present in the form of dietary fibres (Paradiso et al. 2018). The macronutrient profile of ten microgreens was reported by Ghoora et al. (2020). They found that total protein was in the range of 1.81–4.44 g/100 g (FW, fresh weight basis) while total dietary fibers levels were between 1.41 and 3.63 g/100 g FW. Soluble dietary fiber constituted 0.7–16% of total dietary fibers among all varieties studied. Coincidentally, the highest proteins and dietary fibers were observed in fennel and sunflower microgreens out of the ten verities included in the study.

The differences in protein and dietary fibers can be attributed to variety of the microgreens. Similar variations in macronutrient profile were reported by Paradiso et al. (2018). They reported a low amount of sugars, with high fiber and protein content. The protein and fiber content ranged from 1.9 to 3.0 g/100 g FW and 0.06 to 0.62 g/100 g FW, respectively. Coincidently, significant variations in macronutrient profile were seen between microgreens belonging to the same family, but of different cultivars. The lipid concentration ranged from 0.1 to 0.3 g/100 FW, which was very low as compared to proteins and fibers, and almost at par with matured leafy greens.

2.2 Mineral Content

Ash content forms a significant part of microgreen composition as demonstrated by Ghoora et al. (2020) and Paradiso et al. (2018). Ash content ranged between 0.48 and 1.67 g/100 g for the ten microgreens of different varieties studies by Ghoora et al. (2020), with fennel, spinach and carrot having the highest concentration of ash content. Ash content constitutes the mineral elemental composition of food. This includes macro and microelements needed for plant and human growth. The

macro-elements present in foods are potassium (K), phosphorous (P), calcium (Ca), iron (Fe), magnesium (Mg), copper (Cu), manganese (Mn), and zinc (Zn). The elemental composition of microgreens can vary based on the family and variety of the microgreens (Ghoora et al. 2020; Kyriacou et al. 2019a; Xiao et al. 2016), and can also be influenced by both external circumstances. Geochemical profile of microgreens can be significantly altered by extrinsic factors such as growing environment, composition of nutrient solution, type of growing substrate, stage of harvest (Kyriacou et al. 2016). The harvesting stage, especially, has the most significant effect on the mineral content. This is due to the fact that changes in the radical composition during different stages of growth are highly significant (Pinto et al. 2015).

Kyriacou et al. (2019a) examined the differences in mineral content of microgreens of 13 different kinds from *Apiaceae*, *Brassicaceae*, *Chenopodiaceae*, *Lamiaceae*, and *Malvaceae* families. Among the examined macronutrients, K was the most abundant with concentration falling between 26.2 and 68.7 g/kg (DW, dry weight basis). Swiss chard microgreens had the highest amount of K and Na. Other macroelements were also reported, but their concentrations were significantly lower as compared to K, Ca, P, Mg, S and Na, and fluctuated between 1.9 to 32.7 g/kg (DW basis), 4.8 to 8.5 g/kg (DW basis), 5.7 to 9.0 g/kg (DW basis), 1.6 to 10.2 g/kg (DW basis) and 0.9 to 9.8 g/kg (DW basis), respectively. K was also reported the most abundant mineral present among all the samples (Xiao et al. 2016). Its concentration fluctuated between 176 and 387 mg/100 g (FW basis), followed by P and Ca, concentration fell between 52 and 86 mg/100 g (FW basis) and 28–66 mg/100 g (FW basis), respectively. Other notable minerals were Mg and Na with concentrations of 28–66 mg/100 g (FW basis) and 19–68 mg/100 g (FW

basis). The highest potassium content was observed in wasabi microgreens, while red mustard exhibited highest potassium content. 99.7% of the total mineral concentration was represented by the macroelements, namely Ca, Mg, P, K and Na, with potassium being the most abundant element in all microgreens studied (57.1%). 66% of the microgreens samples exhibited phosphorus as the second most abundant element while it was calcium in the remaining 33% of the microgreen samples. Iron turned out to be microelement present in highest concentration (0.47–0.84 mg/100 g, FW basis) in all microgreens with the highest amount seen in rapini (*Brassica rapa*) microgreens. It also accounted for half of the total microelement content in all samples of microgreens. Rapini also had the highest amount of zinc and copper. Lead and cadmium were not detected in any of the microgreens varieties studied. Despite belonging to the same family of microgreens, rapini microgreens exhibited the highest amount of macroelements and microelements among the 30 Brassicaceae microgreens studied (Xiao et al. 2016). K has been repeatedly reported as the microelement with the highest concentration in microgreens (Di Gioia et al. 2019).

Although, the mineral profiles of commonly used microgreens are readily available, a thorough comparison of the mineral profile of microgreen and their mature counterparts is still not abundant enough. Waterland et al. (2017) compared the geochemical profile of microgreens from three cultivars of kale, namely Dwarf Blue Curled, Scarlet, and Red Russian. Five stages of development—fully expanded cotyledon (MG1), seedlings with two true leaves (MG2), seedlings with four true leaves (BL1), seedlings with six true leaves (BL2), and mature plants with more than eight true leaves (adult), were compared with each other. The ash content decreased from 14.6 to 19.1% to 3.9 to 6.4% from MG1 to adult stage. MG1 stage exhibited the

best mineral profile as compared to any other stage on DW basis. However, on FW basis (as consumed), fresh baby leaves exhibited the maximum concentration of minerals. No significant difference was observed between the mineral content of adult produce and MG1 and MG2 stage microgreens. In fact, microgreen stage generally exhibited lower levels of minerals such as K, Ca, Mg, Fe, and Zn as in comparison to BL1 and BL2 stage. Adult kale exhibited higher levels of calcium (Ca) and magnesium (Mg) compared to the MG1 and MG2 stages, which, in turn, had higher levels of sodium (Na). However, lettuce and broccoli microgreens exhibited exactly the opposite trend (Pinto et al. 2015; Weber 2017). They noted a Considerable increase in the mineral content, including Ca, Fe, Mg, Mo, Se, and Zn in lettuce microgreens compared to fully grown lettuce. Broccoli cultivated using compost and hydroponic techniques displayed elevated levels of minerals, including Mg, Mn, Cu, and Zn, in comparison to mature broccoli plants.

2.3 Vitamins and Phytochemicals

Concentrated vitamins are a key advantage to microgreen consumption. The miniature greens are known to be particularly rich in Vitmains C, E and K, as well as several B vitamins including riboflavin, folate, and niacin (Gao et al. 2021b; Marchioni et al. 2021; Meas et al. 2020; Vaštakaite et al. 2017; Xiao et al. 2019). Several studies have been done to compare the levels of vitamins in different varieties of microgreens, and even compare the levels to their mature counterparts. Di Bella et al. (2020) compared ascorbic acid, polyphenols, and antioxidant activity in two traditional Sicilian broccoli microgreens, sprouts, and baby leaves. They concluded that the microgreens stage has the highest ascorbic acid levels (7.5 mg/g) and microgreens also had higher antioxidant activity than baby leaf stage. Concentration of vitamin-C in

nine different varieties of microgreens were estimated by Yadav et al. (2019). The researchers discovered vitamin-C levels in jute and cucumber microgreens was significantly more than their respective fully developed equivalents. Specifically, every 100 of fresh jute microgreens contained 25 mg of vitamin-C, while cucumber microgreens contained 34.90 mg of vitamin-C. In contrast, every 100 g of fresh mature jute plants had 17.45 mg of vitamin-C, and the concentration in mature cucumber plants was 10.00 mg of vitamin-C. However, they found that the remaining six varieties, including amaranthu, bottle gourd (*L. siceraria* Standl), palak (*B. vulgaris* L. var. *bengalensis Roxb*), pumpkin (*C. moschata Duchesne*), and radish, had lower levels of vitamin-C compared to the mature plant. The only exception was water spinach, where both the mature plant and microgreen had similar levels of vitamin-C. Following a similar trend, Ghoora et al. (2020) found that microgreens of fenugreek, spinach, and roselle had much higher vitamin-C content in comparison to their fully grown equivalents. The vitamin-C content in the microgreens was reported to be 120%, 127%, and 119% higher, respectively. The vitamin-C concentration of microgreens in this study varied from 29.9 to 123.2 mg/100 g FW, which is on par with the vitamin-C levels reported in citrus fruits (Khosravi and Asadollahzadeh 2014).

Xiao et al. (2012) conducted a study that profiled the phytochemicals in 25 commercially sold microgreens. They discovered that the levels of vitamin C for every 100 g of fresh microgreens varied between 10.4 and 147.0 mg, which is a notable increase as compared to their fully developed equivalents. The maximum levels of total ascorbic acid were observed in red cabbage microgreens measuring 147 mg/100 g of fresh weight. This was followed by garnet amaranth with 131.6 mg/100 g of fresh weight, and opal basil with 90.8 mg/100 g of fresh weight. This

study also examined the levels of carotenoids, phylloquinone, and tocopherols. The amounts of pigments such as β-carotene, lutein/zeaxanthin, and violaxanthin in the 25 microgreens analyzed varied from 0.6 and 12.1, 1.3 and 10.1, and 0.9 and 7.7 mg/100 g FW, respectively. The concentration of phylloquinone varied between 0.6 and 4.1 µg/g FW, whereas α-tocopherol and ɣ-tocopherol levels were between 4.9–87.4 mg/100 g FW, and 3.0–39.4 mg/100 g FW, respectively. Carotenoids were detected in microgreens from red cabbage, cilantro, crimson amaranth, and green daikon radish. Meanwhile, microgreens of red sorrel, cilantro, red cabbage, and peppercress microgreens were concluded to be rich in β-carotene, with concentrations of 12.1, 11.7, 11.5, and 11.1 mg/100 g of fresh weight, respectively. Additionally, the concentrations of lutein/zeaxanthin in cilantro, red sorrel, red cabbage, and garnet amaranth microgreens were higher than those in other microgreens, with values of 10.1, 8.8, 8.6, and 8.4 mg/100 g FW, respectively. In addition, they conducted a comparison of the phytochemical levels in microgreens with those found in mature vegetables listed in the USDA database. The results indicated that microgreens contained 2–25 times higher amounts of phytochemicals.

Niroula et al. (2019) assessed the chlorophyll and carotenoid composition of wheat and barley microgreens cultivated for a period of 7–16 days. The levels of carotenoid in wheat microgreens elevated to 53.36 mg/100 g DW from 0.42 mg/100 g DW present in the seeds after germination and 16 days of subsequent growth. Microgreens with minimal growth also showed a significant increase in carotenoid content, varying between 0.78 and 56.08 mg/100 g DW. The highest rate of pigment accumulation occurred between seventh and tenth days in wheat and tenth and 13th days in barley. Highest concentration of chlorophyll recorded was

616.63 ± 18.45 mg/100 g DW on 16th day. Additionally, they determined that the carotenoid levels were similar to those seen in carrots, but the chlorophyll levels were slightly lower than kale and similar to spinach.

Kyriacou et al. (2019b) examined the concentration of phytochemicals (chlorophyll, lutein, β-carotene) in 14 different varieties of microgreens. The levels of several phytochemicals exhibited significant variation among the microgreens, even within microgreens of the same species. The concentration of lutein in fresh mustard and jute microgreens was 193.5 mg/kg FW to 827.9 mg/kg, respectively. Fresh kohlrabi had a β-carotene concentration of 426.1 mg/kg, whereas fresh green basil had a value of 8592.2 mg/kg. Tatsoi and pak choi had a greater concentration of chlorophyll in comparison to other microgreens. The anthocyanins, carotenoids, ascorbic acid, and total polyphenol content of Brassicaceae microgreens were assessed by Marchioni et al. (2021). Fresh mustard microgreens had significantly greater levels of anthocyanins (405.53 µg/g) compared to fresh broccoli, daikon, watercress, and rocket salad, which had concentrations of 172.51, 57.56, 52.29, and 42.26 µg/g, respectively. Highest concentration of total polyphenols was found in fresh broccoli microgreens (3.63 µg/g), lowest polyphenols levels were found in fresh mustard microgreens (1.02 µg/g).

2.4 Antinutrients

Antinutrients in plant-based foods are chemicals or molecules that hinder the absorption of other nutrients in the diet, leading to decreased nutrient intake, digestion, and utilisation, as well as the occurrence of undesirable consequences (Galieni et al. 2020). Microgreens have lower levels of antinutrients compared to when they are fully grown. This makes them a desirable addition to the diet of health-conscious individuals as a functional food. Various

methods, either alone or in conjunction, can be employed to effectively manipulate the levels of antinutrients in microgreens. The chickpea microgreens that were 48 h old and produced under blue and red lights had the lowest levels of phytic acid and polyphenols, as revealed in a study conducted by Khattak et al. (2007). In the research published by Giménez et al. (2021), they reported that purslane microgreens grown for 21 days while being exposed to saline conditions (with 80 mM NaCl) exhibited increased fresh weight. Phytic acid content in the microgreens also reduced when they were exposed to LEDs light with blue + red or blue + red + FR combinations with photosynthetic photon flux density (PPFD) of 150 µmol m^{-2} s^{-1}.

Oxalic acid is a prevalent anti-nutritional compound found in green leafy vegetables. It disrupts the activity of cations like calcium and iron by creating insoluble salts. Oxalates that are not bound to other substances bind with calcium ions to create calcium oxalate leading to functional hypocalcemia owing to the insolubility of calcium oxalate. Oxalates also have the potential to precipitate in urine, resulting in the formation of kidney stones, which poses a threat to human health. The oxalate content of microgreens from several vegetables, legumes, oilseeds, and herbs was studied. Researchers discovered that microgreens had significantly lower amounts of oxalate, ranging from two to three times less, compared to their mature leaves (Ghoora et al. 2020). It is noteworthy that microgreens have a very low presence of antinutritional components such as nitrate (NO^{3-}) and nitrite (NO^{2-}) (Sanyukta et al. 2023; K. Yadav 2022). Nutrient deprivation before harvest (DBH) was studied in its relation to the nitrate levels in lettuce mustard, lettuce, and rocket microgreens by Kyriacou et al. (2021). The nutrient solution was substituted with osmotic water for 6–12 days to encourage nutrient deprivation.

Depriving the organisms of nutrients successfully decreased the amount of nitrate; however, the period of the treatment that was effective varied among different species. El-Nakhel et al. (2021) found that introducing moderate salinity, imbalanced mineral levels, or complete nutrient/mineral deprivation in hydroponic growth system of microgreens be beneficial in reducing the presence of anti-nutrients like nitrate.

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3 Production Methods

A diverse range of premium microgreens is cultivated on a commercial scale, while some individuals also grow them on a smaller scale for personal consumption. Growers also engage in mixed cultivation of microgreens. Ensuring the optimal combination of microgreens at the precise moment of harvest is a crucial production strategy for achieving success. The duration from sowing to reaping varies significantly across different crops. The growers can either choose crops with uniform rate of growth while planting a combination of crops in one area, so that the entire crop can be harvested simultaneously with ease. Or they choose to Combine different crops are they were grown and harvested from different areas. Microgreens can be grown in garden beds, window-sills, or containers, depending on the specific needs. The plants are cultivated in a conventional, sterile, porous soil, and several combinations containing peat, vermiculite, perlite, and bark have been proven effective (Mir et al. 2017). Vertical farms offer an efficient method for cultivating them on a vast scale.

Microgreen seeds can be sown either in rows or using a broadcast approach. Many cultivators sow densely in order to optimise yield, but excessive density can lead to lengthened stems and heightened susceptibility to diseases. Most microgreens demand minimal or no fertilizer input, as the seed supplies sufficient nourishment for the young

plant. Nevertheless, the utilisation of different types of fertilisers (albeit in small quantities due to the short growing period), especially organic ones, predominantly enhances the productivity of microgreens.

The choice of substrates for microgreen cultivation has a considerable impact on the nutrient concentration of fresh produce. Vermicompost cultivation led to a higher concentration of minerals in cabbage microgreens as compared to hydroponic cultivation. The properties of various microgreens were enhanced by exposure to abiotic stimuli during the growth period (Ebert 2022). The abiotic stimuli used in the study consisted of thermal stress (40 °C for 2 h), UV-B radiation, osmotic stress (exposure to NaCl), and oxidative stress (exposure to H_2O_2). This led to microgreens with elevated levels of polyphenols and subsequent elevation of their antioxidant activity. Similar study was conducted by El-Nakhel et al. (2021) where they naturally increased the content of functional compounds (ascorbate, plant pigments, and polyphenols) with abiotic stress. They, however, used imbalanced mineral nutrition, low levels of salt or completely deprived the growth solution of nutrients while cultivating microgreens in a soil-less culture system.

Efforts have also been made to cultivate microgreens using hydroponic methods. Nevertheless, microbiological safety is a major concern for microgreens grown hydroponically as compared to the ones grown on alternative substrates. Radish microgreens cultivated in hydroponic systems had higher counts of E. coli O157:H7 in edible as well as inedible portions compared to those grown on peat substrates. The presence of Norovirus in microgreens is due to the efficient uptake of the virus through the roots when it is introduced into the circulating water. Additionally, it is challenging to prevent the cross-contamination in circulating water used in hydroponics,

further contributing to the presence of the virus in the edible tissues of microgreens. Nevertheless, the impacts of these effects will vary across various plant species (Wang and Kniel 2016; Xiao et al. 2015). The importance of standardization in hydroponic systems, particularly in relation to fertiliser, temperature, and light requirements was also highlighted by Bulgari et al. (2017).

Du et al. (2022) explored the potential of hydrogels as a new approach for cultivating microgreens. They suggested that hydrogels could replace traditional substrates, while offering benefits such as reduced water usage by decreasing the need for frequent watering. Additionally, hydrogels can hold and trap molecules of fertilizer and antimicrobial compounds in their matrix, which can be released a slow rate, over a long period of time. This can create a growing environment which is nutrient dense and pathogen free for the microgreens and reduce the consumption of fertilizer and antimicrobial compounds. Hydrogels are made with biopolymers which can be intrinsically altered in their chemical composition to have antimicrobial moieties or low weight bioactives beneficial for microgreen growth. For example, using hydrogels pre-immersed with essential oils for growing microgreens can serve as a novel growth substrate for microgreens. It prevented microbial infestation in microgreens by slowly releasing the antimicrobial compounds during the growth cycle. Additionally, hydrogels can also be used to improve the mineral content in microgreens by being us an immobilizing agent. Minerals, especially trace elements, can be trapped in hydrogels and be effectively absorbed by microgreens during their growth. Elements that can potentially enhance microgreen seedling germination and growth can also be immobilized to aid better microgreen growth. Hydrogels apart from being structurally sound, can also be reused and thus reduce the overall quantity of growth substrate needed.

4 Pre-harvest Interventions

Pre-harvest technologies are aimed to enhance the quality of microgreens while minimizing the risk that comes with consumption of leafy crops. They can improve the nutritional profile, increase yield, and eliminate pathogen to reduce/remove safety hazards. The two most common pre-harvest interventions for microgreens are seed treatment and artificial lighting source.

4.1 Seed Density and Treatments

Seed treatments are an integral part of agriculture and are naturally for microgreen production despite their short growing period. Efficient growth of microgreens is a function of seed quality and germination. Seed density and treatment is of particular interest since it can affect the germination rate and thus the final harvest quantity and quality, respectively. Seed density is a function of seed size as exhibited by (Johnny's Selected Seeds 2017). The study compared 29 microgreen varieties. They experimented with different seed densities for different microgreens depending on the seed size (10–12 seeds/inch^2 for seeds of smaller size and 6–8 seeds/inch^2 for seeds of larger size). The mean densities to obtain maximum yield for basil and radish varieties were in the range of 50 g/m^2 and >200 g/m^2, respectively. Nolan (2019) conducted a similar study where he compared seed densities for seed blends of mustard, basil, radish, and carrot varieties. Basil and radish blend appeared to be most affected by seed density as opposed to arugula and mustard blend. Radish blend showed an increase in the fresh weight yield when seed density was in between 100 and 125 g/m^2. At seed density below 90 g/m^2, the yield decreased. Basil showed similar high fresh weight yield between seed densities of 37–49 g/m^2. However, for both the varieties, the data between

seed density and fresh weight yield showed no statistical correlation. Murphy and Pill (2010) compared seed densities for growing arugula (*E. vesicaria* subsp. *Sativa*) and concluded that sowing rate of 55 g/m^2 resulted in maximum fresh shoot weight as compared to sowing rates with lower density. Ntsoane et al. (2023) reached similar conclusions while measuring the yield of radish, cabbage, and rocket microgreens when grown with varying seed densities. Instead of g/m^2, they calculated seed density with number of seeds per cavity in seedling trays. 4, 8 and 12 seeds per cavity were tested for each microgreen and 12 seed per cavity resulted in highest fresh weight yield for all three. Murphy and Pill (2010), while experimenting the sowing density of arugula microgreens, observed that higher shoot weight (fresh) per m^2 was observed with a sowing density of 55 g/m^2 after 10 days of microgreens growth as compared to lower sowing densities. However, the individual weights of shoots were lower.

A higher seed density, however, doesn't always translate to a higher yield or higher profits. A high sowing rate increases the seed costs and can also lead to fungal infections that can damage the attributes of the final product (Burdon and Ci-Iilvers 1975). Sowing density is a function of several factors and there isn't a standardized approach for all varieties of microgreens. Seed size can vary according to the crop variety and even vary among cultivars and species. Seed production methods can lead to changes in seed size (Bulgari et al. 2017). Thus, a trial-and-error approach is needed to resolve the challenge and fill the knowledge gap.

Seed treatment is another factor that is essential to maintain the quality and quantity of microgreen yield (Murphy and Pill 2010). The simple seed treatment of soaking the seeds to remove damaged or lower quality seeds can also enhance the yield. Pre-germinating the

seeds before sowing resulted in a 21% increased shoot fresh weight while growing arugula microgreens (Murphy and Pill 2010). The germination was achieved by soaking the seeds in water and vermiculite for 1 day and 20 °C. The soaking time can vary depending on the seed size. Larger seeds would need a longer soaking time while smaller seeds would need lesser time (Nolan 2019).

4.2 Effect of Light

Quality of microgreens, although is generally superior to the mature vegetables in terms of concentration of phytochemicals, gets further improved by application of light. There has been concrete evidence of light properties influencing various species of microgreens for the better quality. Work has been to evaluate the impact of light intensity (PPFD), wavelength, photoperiod duration, and frequency (pulsed light and continuous light) on microgreens quality.

4.2.1 Artificial Light Sources

Although microgreens can grow very well in natural light (Dalal 2016; Dalal et al. 2020b), artificial lights give an advantage of growing them indoor without any negative effects from the natural elements. Artificial lights also provide the added advantage of having a higher yield per m^2 of area by using stacked cultivation. The first study on application of artificial lights for microgreen production was done by Kopsell et al. (2012). They hypothesized that level of phytochemicals such as carotenoids and antioxidants in mustard microgreens can be increased by light management. High-pressure sodium (HPS) light bulbs with cold white (160 W) and incandescent (60 W) fluorescent light were used as light source for the microgreens. The photoperiod for both light sources was set at 14-h with photon flux density (PFD) of

275 µmol/m^2 s^1. The PFD was increased to 463 µmol/m^2 s^1 after emergence of first true leaf and applied for 36 h when the microgreens were harvested. This led to the reduction in levels of chlorophyll a and b owing to the high stress levels faced by the growing microgreens. The levels of neoxanthin and β-carotene levels also declined. An increase in zeaxanthin and antheraxanthin was observed while lutein concentration remained unchanged.

On spectral composition analysis, it was found that majority of the light emitted by the HPS lamps was in the yellow and orange spectrum, with small amount of peaks in red, blue, and green regions as well (Zhou and Singh 2002). Thus, the research approach moved to utilization of Light emitting diode (LED) lamps due to their flexibility in producing light from only desired wavelengths (Samuoliene et al. 2012; Brazaitytė et al. 2018). Shift from HPS and LED light also took place since HPS lamps consumed more energy to operate, eventually increasing the overall costs. In addition, HPS lamps generated thermal radiation and their surface exhibited high temperatures (Kopsell et al. 2014; Brazaityte et al. 2015; Craver et al. 2017). This necessitated an increased distance between the plant and lamp to avoid plant burn (Morrow 2008) reducing the output per m^2 of growing area.

Kopsell et al. (2014) and (2017) weighed the changes in broccoli microgreens when grown with HPS lights and LEDs. They primarily overserved that HPS lights had lower fresh shoot weight and lower concentration of plant metabolites. Vaštakaitė-Kairienė et al. (2020) also repeated the experiment of comparing HPS and LEDs to evaluate the changes in nutritional profile of *Brassicaceae* microgreens. HPS lamp (150 µmol/m^2 s^1) was chosen as control and LEDs at PFD of 150 and 250 µmol/m^2 s^1 were compared. Red pak choi (*B. rapa* var. chinensis 'Rubi F1'), tatsoi (*B. rapa* var. rosularis) and mustard (*B. juncea* L. 'Red Lion')

microgreens were grown with LED lights of varying wavelengths. The photo-physiological response of microgreens to LEDs with varying wavelengths differed among the microgreens. Smaller hypocotyls and higher levels of phytochemicals (phenols, ascorbic acid, flavanols, and anthocyanins) were observed in microgreens exposed to LED 150 and LED 250 (Vaštakaitė and Viršilė 2015). They also exhibited a better mineral profile with higher levels of Ca, Fe, K, Mg, Na, P and Zn. The increment in phytochemicals and minerals was direct result of the photooxidative changes that took place in the microgreen tissues because of light treatments. All microgreens also exhibited a higher DPPH (2,2-diphenyl-1-picrylhydrazyl), and ABTS (3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging activity which pointed towards oxidative stress. Significantly lower nitrate content was observed with LED 150 treatment.

The introduction of LEDs thus can be considered as an important factor in the taking the microgreen production to large commercial scale. The key advantages of LEDs are: (i) they emit light in a narrow spectral range; (ii) operate at low voltage; (iii) energy efficient leading to low energy consumption and costs; (iv) photosynthetic photon flux density (PPFD) can be easily regulated for desired plant growth; (v) less heat production and emission during operation which reduces the risk of leaf burn (Pescarini et al. 2023).

4.2.2 Light Intensity and Wavelength

Light intensity or PPFD and its effect on microgreens have been a topic of much interest lately. PPFD is a measure of photon flux present in the light and can change for a given wavelength. PPFD at the same wavelength can have completely opposite effects on plants, including microgreens (Samuoliene et al. 2013). For example, a low

PPFD (>110 µmol m^{-2} s^{-1}) can reduce the photosynthetic rate (Ilieva et al. 2010) and nitrate accumulation (Anjana et al. 2009), while an increased PPFD (<545 µmol m^{-2} s^{-1}) elevated the antioxidant activity in plants, by inducing mild photo-stress that activated the photo-protective mechanisms responsible for primary and secondary metabolite production (Samuoliene et al. 2013).

Samuoliene et al. (2013) studied *Brassicaceae* microgreens for their response to changes in PPFD with respect to their growth and nutrient profile. They subjected the microgreens to light of 455, 638, 665 and 731 nm wavelengths at multiple PPFDs. Lower PPFD (110 µmol m^{-2} s^{-1}) resulted in microgreens of decreased nutritional value and increased nitrate levels in microgreens. In addition, microgreens had lower dry mass due to suppressed growth and a consequent elongation of the hypocotyl. The same microgreens when treated with a mid-rage PPFD (330–440 µmol m^{-2} s^{-1}) showed an increased anthocyanin and thus antioxidant capacity, and a higher leaf surface area along with significantly reduced nitrate content. However, further increment in light intensity to 545 µmol m^{-2} s^{-1} led to pigment degradation caused by oxidative photo-stress. The PPFD did not lead to noteworthy positive change in the microgreen growth and their nutritional quality. This study, however, only considered *Brassicaceae* microgreens. Subsequently, Jones-Baumgardt et al. (2021) researched the influence of PPFD (100–600 µmol m^{-2} s^{-1}) with red (85%) and blue light (15%), respectively on arugula [*E. vesicaria* subsp. *sativa* (Mill.) Thell.], cabbage, 'Red Russian' kale [*B. napus* L. subsp. *napus* var. *pabularia* (DC.) Alef.], and 'Ruby Streaks' mustard [*B. juncea* (L.) Czern] The parameters assessed were morphological feature, growth, and phytochemical profiles. All microgreens exhibited a proportional increase in total and reduced ascorbate concentration, total

phenolics levels, and anthocyanin content with an increase in PPFD. Chlorophyll concentration, however, increased with high PPFD only in kale microgreens and decreased in the other three varieties studied. Xonti et al. (2020) studied the changes in phytochemicals in radish (*Raphanus raphanistrum*) and kale (*Brassica oleracea*) microgreens due to PPFD. They observed that cabbage and radish microgreens when exposed to light with intensity of 270 µmol m^{-1} s^{-1} exhibited stunted growth despite having higher levels of antioxidants. Moreover, kale showed an increased ascorbic acid content when exposed to light with higher intensity (270 µmol m^{-2} s^{-1}).

Vetchinnikov et al. (2021) researched the influence of PPFD levels from 50 to 200 µmol m^{-2} s^{-1} on growth and phytochemical levels of radishes, cabbage, and basil microgreens. Blue and red LED lights of 450 and 660 nm, respectively, were used for a 16-h photoperiod. Plant growth decreased with light intensity greater than 100 µmol m^{-2} s^{-1}. It was thus deemed as the optimum intensity of light to achieve the maximum yield in basil while cabbage and radish thrived best when subjected to 50 µmol m^{-2} s^{-1}. Gao et al. (2021a), similarly, evaluated broccoli microgreens when exposed to LEDs with PPFDs of 30–90 µmol m^{-2} s^{-1} with equal ratios of red, green, and blue lights. They observed that while microgreens exposed to 50 µmol m^{-2} s^{-1} light exhibited maximum yield with higher fresh weight, but the lowest concentration of phytochemical content. The chlorophyll content levels were directly proportional to the light intensity while carotenoids showed inverse proportionality. Microgreens grown with 70 µmol m^{-2} s^{-1} light had highest levels of proteins, sugars, amino acids, flavonoids, vitamin C, and glucosinolates. The changes with light intensity are species defendant as shown by Flores et al. (2024). LEDs of varying intensity (120 ± 5.1, 160 ± 3.6, and 210 ± 5.9 µmol

m^{-2} s^{-1}) were utilized for growing *Brassicaceae* microgreens. Except green and red kale and green cabbage, all microgreens showed increased dry matter content with high intensity light. Low intensity light, however, led to longer hypocotyls in all the microgreens and additionally enhances the cotyledon area in mizuna. The chlorophyll a and b levels showed significant increase with low intensity light, except for red kale and mustard. Antioxidant activity and phenolic content in all microgreens, conversely, showed maximum concentration with high intensity light.

4.2.3 Light Wavelength

While light intensity integral to microgreen growth, light quality is also equally important. Chlorophyll and carotenoids, the two major metabolic molecules in plants, need blue and red light for their optimal functioning. Blue light is essential for hypocotyl growth and cotyledon expansion (Franklin 2016). Additionally, it is essential for phototrophic responses of leaf expansion and leaf positioning, stomatal opening and stomatal conductance, leaf mass proportion, and chlorophyll per leaf area (Franklin 2016; Hogewoning et al. 2010; Ilieva et al. 2010; Jones-Baumgardt et al. 2021; Kami et al. 2010; Withrow et al. 1957). Red light, on the other hand, is integral to adequate growth and development processes in plants, such as germination, cotyledon opening and expansion, inhibition of hypocotyl elongation and petiole growth, and biomass accumulation (Kami et al. 2010). Plant parameters such as growth, yield, and nutritional profile are also indeed a function of quality of light used for the light used their growth (Brazaitytė et al. 2016, 2021). These responses to quality of light are also specific to the species of the crop grown, as demonstrated by (Graham et al. 2019). They tested the hypothesis on six crops grown under

monochromatic and dichromatic red and blue light. While photosynthesis depends on light intensity, photoresponse depends on light wavelength and so does the shoot tissue pigment concentrations (Kopsell and Sams 2013; Kopsell et al. 2014, 2017; Samuoliene et al. 2016). Light in red and blue spectrum leads to changes in the pigment concentration in the microgreen shoot tissues and thereby play an integral role in photosynthesis optimization. The wavelength absorption spectrum differs between plant pigments. Chlorophylls absorbs red (663–642 nm) and blue (430–453 nm) while carotenoids and lutein absorb light in the blue (448–454 nm) region only. Thus, the light wavelength can significantly change the phytochemical composition of microgreens.

Samuoliene et al. (2012) evaluated the effect of light quality on ten microgreens species. Supplemental red light was used 3 day before harvest. 45.1% increase in the anthocyanin production was observed in broccoli (45.1%), followed by 44% increase in kale, 38% in amaranth, 34.5% in tatsoi, 27% in parsley, and 14.6% in pea microgreens when exposed to red light (638 nm). Borage microgreens, in contrast, showed a 51.8% reduction in anthocyanin production followed by 45.1% and 43.3% reduction in mustard and beet, respectively when exposed to red light (638 nm). The total phenols levels in all microgreens (except amaranth) increased with supplemental red light. The increase ranged from 40.8% in tatsoi to 9.1% increase in mustard, except in amaranth it where a 14.8% reduction was observed. As for ascorbic acid concentration, highest increase of 79.5% was observed in amaranth, followed by 65.2%, 60.6%, 59.1% and 25% increase in pea, kale, broccoli, and mustard, respectively. Basil and borage on the other hand exhibited a 53.9% and 46.9% reduction in ascorbic acid content. The cumulative effect of changes in phytochemicals resulted in changes in antioxidant activity of microgreens as well. Highest levels of antioxidant

activities were observed in pea followed by broccoli, borage, mustard and amaranth microgreens. Tatsoi microgreens exhibited the lowest antioxidant activity followed by beet and parsley. The amount of increment in phytochemical concentration was however species dependent and every species did not show a similar increase. Red light, in addition to anthocyanin content, also improved the phenol concentration (Li and Kubota 2009) and overall antioxidant activity on (Wu et al. 2007) in baby leaf lettuce and pea microgreens, respectively.

Samuoliene et al. (2016) assessed the changes in two microgreen varieties, i.e., basil and parsley, when cultivated with supplemental light in the far-red spectrum (635 and 665 nm). There was a significant increase in the concentration of α-tocopherol, phenolics, and ascorbic activity with exposure to the far-red light. The increase in phytochemicals also led to an increased DPPH antioxidant activity. The concentrations of β-carotene and lutein, however, were suppressed in basil and β-carotene levels increased in parsley. They concluded that photoprotective mechanism was stimulated by wavelength-dependent reactions which led to photoresponse of antioxidant compounds. Ying et al. (2021), on the other hand, inferred that changes caused by the light quality and quantity on phytochemical composition isn't as completely linear and doesn't follow a specific trend. They cultivated arugula, 'Red Russian' kale [*B. napus* L. subsp. *napus* var. *pabularia* (DC.) Alef.], 'Mizuna' mustard (*B. juncea* L.), and red cabbage (*B. oleracea* L. var. *capitata* f. *rubra*) microgreens. LEDs with varying ratios of red (70–95%) and blue (5–30%) were used a light source, at PPFD of 300 µmol m^{-2} s^{-1}. Exposure to 20% blue light led to highest levels of reduced and total ascorbate in arugula, kale and mustard, but their concentration dipped with 30% blue light. 30% blue light was, however, ideal for achieving maximum levels of

anthocyanin in all microgreens except mustard. Similar positive changes were observed in cabbage microgreen grown under five light treatments: white, red:blue at 5:1, 5:3, and 5:5 ratios, and single red (Luo et al. 2024). They concluded that mixed red-blue light of 5:1 ratio produced the maximum yield with highest hypocotyl length, fresh weight, edible rate, soluble proteins, vitamin C, phenolics, flavonoids, and glucosinolatess.

Changes in physical attributes of microgreens were observed by Brazaitytė et al. (2016) in Brassicaceae microgreens under red light at 638 and 665 nm, blue light at 455 nm and far-red light at 731 nm. The red light used for indoor growth caused increased fresh mass, decreased leaf area and hypocotyl elongation. The changes thus caused differed between the microgreen species. Additionally, as compared to 665 nm light, exposure to 638 nm light promoted superior nutritional quality with a higher concentration of antioxidants such as ascorbic acid and β-carotene. Kyriacou et al. (2019b), alternatively, observed that plant growth variables were favored by a combination of blue (B, 400–50 nm) and red (R, 600–700 nm) light. They experimented with three light combinations: red (90% R, 10% G), blue (10% G, 90% B), and red-blue (45% R, 10% G, 45% B) (G represents green-yellow light between 500 and 600 nm) with four types of microgreens. The efficiency of blue light to stimulate the plant growth was a function of primary metabolism and was superior to that of the other two light combinations. Blue-light also led to the highest concentration of β-carotene and lutein along with maximum lipophilic antioxidant activity in all microgreens, except purslane. This was result of the demand for defense against oxidative stress caused by blue light, which was fulfilled by an increase in the photosynthetic activity for biosynthesis of additional light-quenching molecules. Conversly, red light maximized the lipophilic antioxidant activity and carotenoid content in

purslane microgreen. Total polyphenols showed a marked decrease with red-blue light, while their levels (especially flavonoids) increased when exposed to blue light since they can easily absorb light at shorter wavelengths (blue light). In case of minerals, K and Na showed a marked increase with red and blue light while Ca and Mg levels decreased, even when the overall mineral composition was largely genotype-dependent. The relationship seen in plants and their respective photosynthetic responses to quantity and quality of light was concluded to be species dependent. These results are consistent with those published by Brazaitytė et al. (2021) for mustard (*B. juncea* 'Red Lace') and kale (*B. napus* 'Red Russian') microgreens grown under different B:R (Blue:Red) light ratios: 100%R, 10%B:90%R, 25%B:75%R, 50%B:50%R, 75%B:25%R, and 100%B. They noted reduction in elongation of microgreens and increase in accumulation of macro and micronutrients with increasing the level of blue light. Varying ratios of B:R pointed towards a species dependent change in plant growth variables, such as leaf area, fresh and dry mass, and optical leaf indexes (for reflectance of chlorophyll, flavanol, anthocyanin, and carotenoids). These findings were also supported by Kamal et al. (2020) while studying the response of 21 varieties of Brassica microgreens to light combinations. They inferred that the combination of 20% blue: 10% green: 70% red yielded the best growth while 80% blue: 20% red led to a significant elevation in vitamins and mineral levels in microgreens. Similar results were observed with cabbage, kale, arugula, and mustard microgreens grown with red and blue lights (Ying et al. 2020). Blue light did not affect the fresh and dry yields of the species, except cabbage. The species responses to blue and red lights were markedly species dependent and one size fit all approach isn't the best path for indoor microgreen production.

Most of the research on microgreens and light quality has been conducted with blue and red light with very few studies on green-yellow light (Carvalho and Folta 2016; Gerovac et al. 2016; Craver et al. 2017). Carvalho and Folta (2016) examined the potential of green wavebands in modulating the accumulation of anthocyanin in Red Russian kale (*Brassica napus pabularia*). Green (525 nm) light was tested along with far-red and blue (730 nm and 470 nm, respectively). They concluded that although far-red light induced production of anthocyanins, the green light enhanced the production and accumulation under PPFD. Blue light induced anthocyanin production was, however, inhibited by green light depending on the light PPFD. The final anthocyanin content depended more on the PPFD and the genotype of the species considered as compared to the wavelength. Similar claims were made by Gerovac et al. (2016) and Craver et al. (2017). They discerned decreasing total carotenoids concentration in mizuna (*B. rapa* var. *japonica*) and mustard (*B. juncea* 'Garnet Giant') microgreens and increasing anthocyanins concentration in kohlrabi (*B. oleracea* var. *gongylodes*) microgreens with increasing light intensity. They change in concertation of phytochemical was independent of light quality. Gerovac et al. (2016) also noted that despite changes in light quality, increase in hypocotyl length and dry weight increased in all microgreens was proportional to an increase in the PPFD 315 from 105 µmol m^{-2} s^{-1}. Additionally, kohlrabi microgreen exhibited a marked reduction in leaf area and increment in relative chlorophyll content with increasing PPFD. Lower PPFD also resulted in improved nutritional profile irrespective of the light quality.

Apart from red, blue, green light, amber light in the UV-A and UV-B spectrum have also been established as a potential light source for microgreen growth (Alrifai et al. 2021; Brazaitytė et al. 2019; Gao et al. 2021a; Lu et al.

2021). The research on these light sources is in early stages. Amber light was assessed for its ability to accumulate carotenoids in *Brassicaceae* microgreens in combination with red and blue lights (Alrifai et al. 2020). Total and individual concentration of carotenoids, such as β-carotene, lutein, α-carotene, neoxanthin showed an increase of 20–44% while and violaxanthin increased 10–55% under dose-dependent amber-blue light. The increasing level of red light led to a decrease in the concentration of these pigments. A slight increase in lipophilic DPPH and ferric reducing activity (FRAP) was observed in high amber light and blue light combination. Supplemental application of UV-A LEDs of differing wavelengths (366, 390, and 402 nm) were tested for their affect on growth parameters and phytochemicals concentration of microgreens [basil (*O. basilicum* L., Sweet Genovese), beet (*B. vulgaris* L., Bulls Blood), and red pak choi (*B. rapa* var. chinensis, Rubi)]. Wavelengths of 366 and 390 nm increased the phytochemical concentration of all microgreens, with highest increment observed in pak choi. Supplemental UV-A treatments increased the leaf area, fresh weigh, concertation of phenols, anthocyanins, ascorbic acid, and α-tocopherol. This also led to subsequent increase in the DPPH activity of microgreens. This increase was species dependent. UV-A light also increased nitrate accumulation only broccoli and tatsoi microgreens and decreased the mineral accumulation in all microgreens except mizuna. A very similar study was again conducted with supplemental application of UV-A light only for mustard microgreens (*B. juncea* L. cv. ‘Red Lion’), at 366, 390, and 402 nm and for 10 and 16 h (Brazaitytė et al. 2019). While biomass accumulation wasn’t affected by supplemental UV-A exposure, leaf area increased with 402 nm irrespective of exposure time. Exposure at 402 nm also led to increase in total phenolic and α-tocopherol content

while nitrates concentration showed an increase under 366 nm and 390-nm irrespective of radiance duration. 366 nm irradiance increased the concentrations of lutein/zeaxanthin and β-carotene at 10-h duration while 390 nm showed same results at 16-h duration. Mineral content also increased significantly with UV-A light and at a 16-h irradiance.

Instead of using only UV-A light with normal LEDs, Gao et al. (2021a), used UV-A supplemental light in combination with selenium application for growth of broccoli microgreens. Selenium and UV-A were used individually (Se 100 µmol/L Na_2SeO_3; UV-A- 380 nm, 40 µmol/m^2/s) and in combination, Se + UVA. Foliar Selenium application was done after seeds germinated while UV-A irradiance was observed for 15 min. Both the treatments when used individually or in combination led to an increase in levels of phytochemicals albeit with inhibited plant growth. A significant increase in phenolic compounds, soluble sugars, flavonoids, organic Se, ascorbic acid, and Fe was observed in microgreens with the Se treatment. This treatment also led to reduction in Zn content. Phytochemicals such as chlorophylls and phenolic compounds, soluble proteins and the FRAP antioxidant activity showed a marked increased with the UV-A treatment. The maximum levels of total anthocyanins, glucoraphanin, total aliphatic glucosinolates, and total glucosinolates were observed in the combination treatment. Broccoli microgreens were grown with UV-B lighting and UV-B + calcium chloride foliar spray individually and in combination (Lu et al. 2021). The combination treatment (0.27 Wh/m^2 UV-B and 10 mM $CaCl_2$) showed least tissue electrolyte leakage along with off-odor after harvest. UV-B (0.27 Wh/m^2) treatment significantly hiked the glucosinolate accumulation in microgreens.

4.2.4 Photoperiod and Light Frequency

Brazaitytė et al. (2019) examined the efficacy of photoperiod duration (10 and 16 h) of supplemental UV-A light on growth and phytochemicals in mustard microgreens (*B. juncea* L. cv. 'Red Lion'). UV-A light at three wavelengths, 366, 390, and 402 nm was used. They observed that highest accumulation of minerals, except iron, at 16-h photoperiod of 402 nm light. 10-h photoperiod of 366 nm and 16-h photoperiod of 390 m resulted in increase in concentration of lutein/zeaxanthin and β-carotene. Mlinarić et al. (2020) examined the potency of 24- and 48-h photoperiods antioxidant potential (DPPH and FRAP) of on chia microgreens. They concluded that 48-h exposure significantly hiked phytochemicals levels (phenolics, ascorbic acid, chlorophyll, and carotenoids) and consequently the antioxidant capacity. Moving from chemical to physical attributes, Kong et al. (2019) investigated the efficacy of shortened photoperiod of 16-h instead of 24-h photoperiod on physical attributes in arugula (*B. eruca*, 'Rocket'), cabbage, mustard (*B. juncea*, 'Ruby Streaks'), and kale (*B. napus*, 'Red Russian') microgreens. Blue (440 nm) and red (665 nm) light were used for both photoperiods. The overall hypocotyl length along with the rate of stem extension were better with a 24-h photoperiod under blue light as compared to a 16-h photoperiod. Elongation of hypocotyl was also enhanced by Kong and Zheng (2021) in seedlings from sunflower (*H. annuus* 'Black oil') and arugula (*E. sativa* 'Rocket'). The seedlings were grown in dark until day 5 followed by light exposure for 7 days and 2 days for a 24-h photoperiod using combination light (R:B of 85:15 at 100 µmol m^{-2} s^{-1}). Hypocotyl elongation seen in sunflower (26%) and arugula (28%) was stimulated owing to the dark treatment at early stages of growth. This led to an increase in sunflower yield by 13% whereas the arugula yield was reduced by 24%.

Shibaeva et al. (2022) also focused on the physical attributes of arugula, broccoli, mizuna (*B. rapa*. Var. nipposinica), and radish (*R. sativus* var. radicula) microgreens cultivated. Photoperiods of 16 and 24 h were employed and compared and provided daily light integral (DLI) of 15.6 and 23.3 mol m^{-2} day^{-1}, respectively. Also, LED lamps and fluorescent lamps were used, creating four different combinations of light variants. 16-h photoperiod resulted in higher fresh weight, dry weight, leaf mass per area and robust index in all microgreens, irrespective of light quality. Same four crops were grown under similar conditions by Shibaeva et al. (2023). In this experiment, however, all four microgreens had a better yield with superior nutritional value, including higher levels phytochemicals, such as of anthocyanins, flavonoids, carotenoids, and proline. This led to a subsequent increase in the antioxidant activity along with reduced nitrate content as compared to 16-h photoperiod. Kumar et al. (2023) evaluated the compositional changes in mung bean, chickpea and lentil microgreens, taking place with the changing the photoperiod from 16 to 22 h. The temperature in light and dark conditions was maintained at 22 °C and 17 °C respectively. There was marked increase in the phenolic acid levels with increased photoperiod. The most prominent phenolic acids which showed an increase were chlorogenic acid and p-coumaric acid. A significant increase was also observed in sugar and its derivates with the elongated photoperiod. Physical parameters such as plant height, and phytochemicals such as chlorophyll and antioxidant activity also showed a marked increase with the increased photoperiod.

Apart from photoperiod, light frequency (pulsed light, Hz) is another essential parameter for indoor growth of microgreens. It directly correlates to energy costs and the temperature management to offset the heat generated

during lighting (Song et al. 2019; Vaštakaite et al. 2017, 2018; Vaštakaitė-Kairienė et al. 2020). LED Pulsed lighting system saves energy by emitting short bursts of light rather than a continuous stream of photons and produces less heat which helps with temperature management (Yeh and Chung 2009). Red pak choi (*B. rapa* var. chinensis), mustard and tatsoi (*B. rapa* var. *rosularis*) microgreens were grown under monochromatic pulsed light (Vaštakaite et al. 2017). LEDs of 455, 470, 505, 590 and 627 nm was employed with frequencies of 2, 32, 256 and 1024 Hz at 200 ± 10 µmol m^{-2} s^{-1}. All light frequencies except 32 Hz raised the accumulation of phenolic compounds in mustard microgreens, while anthocyanins accumulated in red pak choi and tatsoi at 2 and 32 Hz. Superior antiradical potential was observed in mustard when subjected to all light frequencies, except 2 Hz. However, the 2 Hz frequency raised the antiradical potential in tatsoi and red pak choi. Similar increase in phytochemicals was observed in basil (*O. basilicum* L. 'Sweet Genovese') microgreens farmed with pulsed light (2, 32, 256, and 1024 Hz) with monochromatic LEDs with supplemental lighting (Vaštakaitė et al. 2018). In case of basil however, the highest accumulation of phenolic compounds and anthocyanins was observed at 1024 Hz with supplemental blue or red light. Light frequency of 32 and 256 Hz resulted in higher ascorbic acid accumulation for basil microgreens.

Mustard (*B. juncea* L., 'Red Lion') microgreens were grown and harvested under similar conditions as basil, but the efficiency of light frequency for mineral accumulation was studied (Vaštakaitė-Kairienė et al. 2020). Blue and yellow light (455 and 590 nm, respectively) at 2 Hz led to the highest levels of potassium. Ca levels increased with all supplemental wavelengths as long as light frequencies were maintained at 32 and 256 Hz. P and S exhibited similar trends of increased concentrations with

supplemental light. Mg, however, showed an opposite trend with reduced concentration with similar growth conditions. Fe and Zn, on the other hand, exhibited slightly but significantly higher levels with 2, 32 and 256 Hz light frequencies irrespective of the wavelength, except blue light. Song et al. (2019), on the other hand, tried to manipulate physical traits of microgreens with pulsed light. 5, 10 and 20 s and 1-h pulses of LED lights were used for growth of red Russian kale, purple top turnip, or ruby queen beet microgreens grown with a 12-h photoperiod. They inferred that photomorphogenic development was hindered with longer pulses (1 h on/1 h off). Short pulse duration of 1 s led to similar growth as constant 12-h photoperiod. Slightly longer pulses of 5 and even 10 s did not affect the growth. This significantly reduced the energy consumption by 30–50% and made the production process energy and cost efficient. Further increase of pulses duration to 20 s however led to small negative changes in the microgreens.

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5 Post-harvest Strategies, Shelf Life and Food-Safety Concerns

Microgreens are a type of fresh produce that have a rapid respiration rate. After being harvested, these plants quickly enter the senescence stage and display a short shelf life due to the abrupt halt in their growth at an early stage. Leaf senescence is characterized by rapid changes in leaf physiology and biochemistry (Lim et al. 2007). Microgreens rapidly degrade their major products due to being harvested at the cotyledon stage with the next step being spoilage if optimum storage and post-harvest conditions are not met (Chandra et al. 2012). Due to their limited shelf life, microgreens must be preserved and handled with great care until they are consumed. Studies have investigated

preharvest and postharvest measures, such as selecting the appropriate time to harvest, using modified environment packaging, controlling temperature, and managing light exposure, in order to preserve quality, enhance and maintain nutrition level, and prolong the shelf life of products.

5.1 Harvesting and Washing with Minimal Injury

Microgreens can be harvested within a time frame of 7–14 days after germination in tropical climates, and during a somewhat longer period of 14–28 days in in temperate climates characterised by cold weather. The specific duration of growth depends on the type of crop and the surrounding environmental factors. Microgreens are characterised by their young and delicate leaves, which consist of either two fully developed cotyledon leaves or the initial set of cotyledon leaves along with partially developed true leaves. The microgreens are available in a range of colours, textures, and flavours. They usually have a height of 2.5–6 cm, which is smaller than baby greens (Allende et al. 2004; Sanyukta et al. 2023; Xiao et al. 2014b).

Rapid quality degradation following harvest is a significant impediment to the expansion of the microgreen industry. Due to their high surface area to volume ratio, high respiration rate, and delicate leaves that are susceptible to physical damage and wilting, storing microgreens is a multi-faceted challenge. Additionally, they are susceptible to rapid postharvest decomposition, have high rate of transpiration, can have leakage of nutrient-rich exudates, tissue damage, and also show early senescence due to high biochemical activity (Turner et al. 2020). After harvest, microgreens may be washed to eliminate soil particulates and ensure a clean product for packaging. The initial bacterial load is reduced by washing greens prior to

packaging. However, this process creates a humid environment that encourages microbial growth and requires the removal of excess water. The delicate greens are more susceptible to microbial growth, and the additional handling that washing and dewatering entails can damage them, which is why many producers opt not to wash them. It is a challenge to eliminate excess moisture after washing without causing injury. While harvesting, it is important to avoid injury, as microgreens that are injured are more likely to harbor pathogens and spoil at a quicker rate and potentially spread the spoilage causing microbes too their neighbors (Aruscavage et al. 2008) observed that traumatically injured leaves harbored populations of E. coli O157:H7, while healthy ones did not. To optimize the quality retention and shelf life of microgreens, while simultaneously discouraging the growth of spoilage microorganisms and human pathogens, a delicate equilibrium must be achieved with respect to temperature, moisture, and atmosphere (Kumari et al. 2023).

5.2 Storage Temperature

Among all the factors that can cause quality changes to fresh produce, temperature is the most important one. Effective temperature management slows down the metabolic respiration in fresh produce, thereby slowing senescence, product decay, and extending their shelf life (Dayarathna et al. 2023; Oliveira et al. 2015; Patil et al. 2024; Saltveit 2020; Yan et al. 2022). Optimal temperature control is crucial for preserving the quality and longevity of microgreens throughout transportation. By effectively managing storage temperatures, microgreens may be transported over greater distances without compromising their nutritional content, freshness, and susceptibility to rot and decay. The cell membrane integrity of microgreens is crucially maintained by low temperature, which also effectively suppresses microbial activity, thereby

preventing spoiling. The degradation rate of perishable items escalates by a factor of two to three for every 10 °C temperature hike (Kader and Solle 2004). Storage temperature of fresh commodity depends on its sensitivity of cold temperature and tissue damage. While most fresh commodities can be stored at temperatures as low as 0 °C, some may show tissue damage leading to tissue electrolyte leakage, increased rate of respiration and rapid onset of senescence. The level of sensitivity to chilling, although determined by genetics, is also influenced by the atmosphere within the packaging and the stage of growth (Kyriacou et al. 2016).

Microgreens once harvested can be stored for 5–10 days at refrigerated temperatures (Mir et al. 2017; Paradiso et al. 2018; Patil et al. 2024). The shelf life can be increased up to 14 days with optimum post-harvest treatments and storage with the right cultivar (Patil et al. 2024; Ranjitha et al. 2023; Yan et al. 2022). Although microgreens do not have any legal storage temperature requirements, studies point towards storage at ≤5 °C (Kou et al. 2013; Xiao et al. 2014b) for slowing down the metabolic processes. Storing them below 1 °C or freezing temperatures can, however, lead to chilling injury, as seen in other leafy vegetables, and can cause physical damage leading to tissue leakage (Turner et al. 2020; Kumari et al. 2023). The storage should also be done at optimum humidity. High humidity can cause fungal growth whereas low humidity can lead to drying of produce (Sandhya 2010). Hence, a comprehensive storage environment w.r.t. temperature, humidity and gaseous composition is needed for microgreen that can ensure low respiration rate, reduce moisture loss and inhibit growth of pathogenic microbes.

In their study, Kou et al. (2014) examined the impact of different temperatures (ranging from 1 to 20 °C) on quality characteristics of packed baby spinach microgreens. Microgreens retained their quality for 18 days after harvest

when stored at temperatures of 1 and 4 °C. However, temperatures above 8 °C were unable to preserve the produce quality. Storing the product at a temperature of 8 °C resulted in a decrease in its visual attractiveness, as well as an increase in the leakage of electrolytes from the tissues, the development of an unpleasant odour, and the yellowing of the product. Xiao et al. (2014a) concluded that storage at 1 °C maintained the post-harvest quality and thus the shelf-life of radish microgreens better than 5 °C storage. Buckwheat microgreens, meanwhile, showed best keeping quality at 5 °C (Kou et al. 2013). This temperature significantly slowed down microbial growth such that the keeping quality was acceptable for 21 days. Yan et al. (2022) also noted that the quality of buckwheat microgreens, in term of their nutritional profile, was only acceptable up to 8 days of 5 °C storage after treatment with organic acids. They observed a sharp decline in phytochemicals such as phenolic and flavonoids and subsequently the antioxidant capacity of microgreens from 8 to 12 days after storage. Furthermore, there was a concomitant occurrence of unpleasant odor, leakage of tissue electrolytes, proliferation of bacteria and fungi, and a general deterioration in the visual appeal of microgreens. Sunflower and broccoli microgreens exhibited a prolonged shelf life of 12 days when stored at temperatures of 10 °C and 5 °C, respectively (Dalal et al. 2019, 2020a; Patil et al. 2024). Paradiso et al. (2018) reported shelf life of chicory, lettuce and broccoli microgreens did not exceed more than 10 days when stored at 5 °C.

5.3 Modified Atmosphere Packaging

Modified atmospheric packaging (MAP) alters the air inside the package to enhance the shelf life of fresh food. This is done by reducing the amount of oxygen (O_2) and increasing the amount of carbon dioxide (CO_2) in the package's

headspace (Oliveira et al. 2015; Saltveit 2020; Sandhya 2010). Typically, maintaining an environment with oxygen levels between 3% and 6% and carbon dioxide levels between 2% and 10% effectively controls microbial growth and increases the lifespan of many fresh-cut items. CO_2 is the gas of significance in MAP due to its direct antimicrobial effect. CO_2 reduces microbial multiplication by affecting the lap phase of microbial growth which control maximum population density that could otherwise be reached (Devlieghere and Debevere 2000). However, this impact on microorganisms is not consistent across the board and is contingent upon factors such as the specific microorganism, its growth stage, temperature, water activity, the pace at which gases can pass through the packaging material, and the nature of the product. MAP, although beneficial to fresh produce, need to be optimized for each fresh commodity since its misuse can cause physiological disorders, fasten senescence in plant tissue, increase susceptibility to pathogen growth and decay and prevent plant wound healing (Day 1993). High concentration of CO_2 can also cause tissue injury in some plant, while low O_2 levels can lead to anaerobic respiration leading to ethanol and acetaldehyde accumulation, thereby producing off-odor and off-flavors (Allende et al. 2004).

The reduction in the oxygen level and elevation in the carbon dioxide level contribute to the elongation of the microgreens' shelf-life. The microgreens are often enclosed in polyethylene packaging with MAP conditions and refrigerated to the necessary temperatures prior to being distributed to the market or consumers. Biodegradable clamshell containers have also been developed for packaging of fresh produce. The oxygen transmission rate (OTR) of packaging materials/films can be used to measure the effectiveness of the packaging films in maintaining the quality of the microgreens (Kou et al. 2013; Xiao et al.

2014a). The first instance of application of MAP to enhance the shelf life of microgreens was reported by Kou et al. (2013). Buckwheat microgreens were enclosed in polyethylene (PE) bags measuring 15 × 15 cm. The bags were made of a film with oxygen transmission rates (OTRs) of 8.0, 16.6, 21.4, and 29.5 pmol/(m^2 s Pa) and stored at 5 °C. The O_2 and CO_2 levels inside the 16.6 OTR bags reached a stable range of 14.8–15.3 kPa for O_2 and 1.1–1.4 kPa for CO_2 by the seventh day of storage. This equilibrium remained unchanged until the conclusion of the 14-day storage duration. Bags fabricated from films with varying oxygen transmission rates (21.4 and 29.5 pmol/(m^2 s Pa)) exhibited comparable trends, albeit with marginally elevated oxygen concentration (16.3–16.8 kPa) and reduced carbon dioxide concentration (0.8–1.2 kPa). Bags fabricated from the film with the most minimal oxygen transmission rate (8.0 pmol/(m^2 s Pa)) sustained reduced levels of O_2 (11.7–13.6 kPa) and elevated levels of CO_2 (1.5–1.7 kPa) from fourth day leading up to the end of storage. Regardless of alterations in the gaseous composition, all microgreens exhibited significant tissue electrolyte leakage on both day 14 and day 21. Therefore, Buckwheat microgreens stored at a temperature of 5 °C in films with an oxygen transmission rate (OTR) of 16.6 pmol/(m^2 s Pa) exhibited optimal performance, as they were able to maintain their highest quality attributes and tissue integrity throughout the entire 21-day storage period. Xiao et al. (2014a) however conducted a similar experiment with radish microgreens and packed them in polyethylene films with OTRs ranging from 8.0 to 29.5 pmol/(m^2 s Pa). They did not observe any noteworthy changes in keeping quality and microbial load of microgreens with changes in the OTR.

Packaging material plays in important role in maintaining the headspace gas composition for microgreens. Chandra et al. (2012) packed '*Tah Tasai*' Chinese cabbage microgreens in 35 µm polyethylene or polypropylene bags. They were stored for 9 days at 5 °C. Headspace O_2 partial pressure of both packages decreased with time. Polypropylene packaging, however, exhibited a higher CO_2 partial pressure in the headspace and increased electrical conductivity in microgreens. The microgreens in polyethylene, on the other hand, exhibited lower off-odor and thus it was more suited for packaging Chinese cabbage microgreens. Ghoora and Srividya (2020), on the other hand used two distinct packaging materials to store radish and roselle microgreens after harvest. The first choice was polyethylene terephthalate clamshell containers (PET–CS) with a hinged lid. The PET-CS containers measured 12.5 × 10 × 3.5 cm in dimensions and had a thickness of 0.2 mm. PET-CS packaging was compared with low-density polyethylene self-seal bags (LDPE–SSB) which measured 12.5 cm in length and breadth with film thickness of 0.14 mm. Microgreens packed in PET-CS containers had lower respiration rate of 41.5 and 45.8 µL CO_2 g^{-1} h^{-1} for radish and roselle microgreens, respectively. Their respiration rate in LDPE-SSB bags was 47.3 and 51.3 µL CO_2 g^{-1} h^{-1}, respectively. The reduced rate of respiration observed in PET-CS resulted in decreased electrolyte leakage and microbial counts, as well as increased overall acceptability of microgreens after 8 days of harvest. The superior performance of PET-CS was ascribed to its reduced water vapor permeability in comparison to LDPE. Yan et al. (2022) on the other hand used different variation of polyethylene to store buckwheat microgreens. Microgreens were stored for 0, 2, 4, 6 and 8 days in three different packaging materials. PE bags with 12 µm thickness, LDPE bags with 38 µm thickness, and high-density polyethylene

(HDPE) bags with 6.8 µm thickness. A rapid reduction in O_2 and an increase of CO_2 was observed in microgreens in LDPE films as compared to PE and HDPE films. The O_2 reduction in LDPE film was rapid in initial 2-day storage, followed by a slower reduction rate till 6 days and again rapid reduction from day 6 to 8. The CO_2 levels, however, reached an equilibrium of 1.5–2.0 kPa in LDPE films 2 days after storage. While LDPE film packed microgreens showed lower tissue electrolyte leakage and weight loss as compared to PE and HDPE, the visual quality, off-odor, and microbial count did not vary significantly between the packaging materials. LDPE was also reported to have better preserve the quality of mungbean (*V. radiata* L. Wilczek), lentil (*L. culinaris* Medikus subsp. *culinaris*), and Indian mustard (*B. juncea* L. Czern & Coss.) microgreens (Priti et al. 2022).

5.4 Postharvest Light Treatments

Light wavelengths, apart from influencing the composition of microgreens during growth, can modify shelf life of microgreen by reducing quality loss and microbial load, improving physiological activity, and delaying tissue softening (Sharma et al. 2023b). Varying proportions of light spectrum during storage can exert distinct impacts on the shelf life of microgreens, which can either be advantageous or detrimental depending on the exact type of microgreen (Gil and Garrido 2020; Haley 2023; Lu et al. 2018; Pintos et al. 2020; Priti et al. 2022; Sharma et al. 2023b; Xiao et al. 2014a). Xiao et al. (2014a) subjected radish microgreens to two storage conditions: dark storage and continuous fluorescent light exposure (light intensity ≈ 30 µmol s^{-1} m^{-2}) for durations of 0, 4, 8, 12, and 16 days. It was observed that microgreens placed in darkness had superior keeping quality compared to those stored under fluorescent light, while the latter caused the

microgreens to deteriorate. According to their findings, exposure to light caused an increase in the amount of ascorbic acid in microgreens, while the amounts of α-tocopherol and total phenolic remained the same. Conversely, storing in a dark environment led to increased ability to remove hydroxyl radicals and better preservation of carotenoids. There were no notable disparities observed in the relative antioxidant activity between the light and dark treatments. Broccoli microgreens, meanwhile, exhibited higher level of glucosinolates under postharvest UV-B radiation (312 nm) (Lu et al. 2018).

5.5 Postharvest Chemical Treatments

Microgreens cultivation and harvesting can introduce microbes to the produce which can cause spoilage and/or diseases. Utilising mild post-harvest treatments for minimal processing of fresh produce shows promise in reducing the presence of microorganisms in microgreens, while preserving their shelf life (Kader and Solle 2004; Chandra et al. 2012; Luo et al. 2024; Dalal et al. 2019, 2020b; Sharma et al. 2023a; Patil et al. 2024). Mild treatments such as organic acids, chlorine wash, electrolyzed water, ethanol treatments and even edible coatings are implemented to boost the shelf-life and maintain safety in microgreens (Bhaswant et al. 2023; Chandra et al. 2012; Dalal 2016; Dalal et al. 2019; Patil et al. 2024).

Although, organic acids used in such treatments inhibit microbial growth, the extra moisture introduced to the microgreens during the treatment must be minimized/removed thoroughly to maintain low moisture in the package. This was demonstrated aptly by other workers (Kou et al. 2013; Xiao et al. 2014b). Buckwheat (Kou et al. 2013) and radish (Xiao et al. 2014b) were washed with chlorine—50 and 100 mg/L and stored for 21 days and 28 days respectively (with unwashed and water wash as control). In both cases, the microgreens that were not

washed had a lower number of bacteria at the conclusion of each storage period compared to the ones that were washed with chlorine. They also did not deteriorate as fast as the washed ones. The first reason for this was the damage to the microgreens during washing and centrifugal dewatering. While storing buckwheat microgreen (Kou et al. 2013), chlorine wash effectively reduced the microbial during the initial days of storage, but after the seventh day, the microbial count rebounded and at 21 days of storage, it far exceeded those of unwashed microgreens. Even the samples washed with water had a higher bacterial load than chlorine washed microgreens than unwashed ones, possibly was due to the extra added moisture after harvest that could not be removed efficiently. In case of radish microgreens (Xiao et al. 2014b), however, 100 mg/L chlorine washed sample exhibited the maximum microbial count by the end of storage period at the 28-day mark. The alteration of the natural microbial flora of the microgreen by the chlorine treatment was cited as the reason for this unexpected increase in microbial load. They hypothesized that radish microgreen must possess a natural microflora that was destroyed by the chlorine wash, and this provided ample opportunity to the chlorine resistant microbes to thrive after effect of chlorine subsided eventually. However, when chlorine treatment was replaced with calcium lactate (50 mM) postharvest dip, different results were obtained in case of broccoli microgreens stored for 14 days (Kou et al. 2015). It significantly reduced the microbial load and the tissue electrolyte leakage in the microgreens.

Organic acids were used to improve the quality and microbial safety of '*Tah Tasai*' Chinese cabbage (*B. campestris* var. narinosa) microgreens stored for 9 days (Chandra et al. 2012). The treatment they tested were chlorinated water (Cl, 100 $mL \cdot L^{-1}$), a citric acid solution

(CA, 0.50% w/v), as well as a combined citric and ascorbic acid solution (CA + AS, 0.25% w/v each), followed by 50% (v/v) citric acid + ethanol spray (CA + E), with tap water wash as control. Post chemical wash, samples were packaged in either PE or polypropylene (PP) bags of 35 µm thickness. Off-odor was under the threshold limit until 9 days storage in CA + AS and CA + E treatments, whereas superior visual attributes were exhibited by CA + AS and CA + E treated microgreens. While chlorine washed samples had lowest microbial load on day 0, it was minimum in CA + E on day 9. They concluded that CA + E treatment worked best to keep microbial load in cabbage microgreens. Additionally, it was determined that using PE film for packaging microgreens would offer greater advantages compared to using PP. Dalal et al. (2020a) conducted a comparable investigation on improving the shelf life (16 days at 10 ± 1 °C) of sunflower microgreens with organic acid sprays. The post-harvest application involved the use of organic acids that are generally regarded as safe (GRAS), specifically citric acid (CA), ascorbic acid (AA), a combination of citric acid and ethanol (CA + E), and a combination of citric acid and ascorbic acid (CA + AA). Distilled water wash was employed as a control. CA + AA, CA, and AA treatments exhibited least microbial load after 16 days of storage unlike in case of cabbage microgreens. Furthermore, these treatments also preserved the highest levels of phytochemicals such as ascorbic acid and total phenols, which consequently led to a higher DPPH scavenging activity. CA + AA treatment exhibited the highest level of chlorophylls and carotenoids, while also demonstrating the lowest tissue electrolyte leakage after 16 days. Control and AA treatments similarly retained high concentrations of total chlorophylls and carotenoids, respectively.

The efficacy of chlorine water and citric acid in preserving the shelf life of Tartary buckwheat microgreens

was examined (Yan et al. 2022). The microgreens were treated with chlorine wash (Cl, 2 mg L^{-1} ClO_2), citric acid wash (CA, 1% citric acid w/v), and Cl + CA, with water wash as control. By the end of 12-day storage, the Cl treatment exhibited the highest microbiological count and tissue electrolyte leakage. However, the combined treatment of Cl + CA yielded the most favourable results for both of these parameters. The visual quality and off-odor scores fell in the same range for all treatments including control. Patil et al. (2024) combined two organic acids for wash treatment to preserve the shelf-life of broccoli microgreens. They used ascorbic acid and citric acid at a concentration of 0.25% w/v each with water wash as control. Instead of applying centrifugal force for moisture extraction after washing, the microgreens were allowed to air dry for 20 min to ensure removal of excess moisture. The organic acid washed microgreen exhibited enhanced storage stability, maintained its phytochemical composition, and exhibited reduced microbial contamination after 16 days of storage.

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6 Tailored Value Added Food Products

Microgreens are becoming increasingly popular as a novel culinary element. They are utilized to augment salads or serve as edible embellishments for a diverse range of cuisines. Microgreens can be used as fundamental components in the creation of both sweet and savory foods (Mir et al. 2017; Renna et al. 2017). Given the quick product deterioration, it is crucial to effectively manage the postharvest of microgreens to maintain their quality, stability of bioactive components, and shelf life for culinary applications. Furthermore, other from being consumed in their raw state, microgreens can also be employed as a

component in the food processing sector. Microgreens provide a wide range of opportunities for developing creative culinary products. These encompass a variety of products such as juices, fermented beverages, breakfast items or snacks, seasonings, such as vinegar or sauces, weaning foods, probiotic foods, yoghurt, dried sprouts and microgreens supplemented flours, baked products, confectionary, and even varieties of tea. Broccoli sprout juice can be extracted by means of mechanical pressing. The clarified juice, obtained through centrifugation at a temperature of 4 °C for a duration of 30 min, can be mixed with different types of liquids. The by-product of juice production, sprouted wheat pomace powder, can be added to flour combinations. The inclusion of this additive was discovered to greatly improve the nutritional content of the wheat flour (Sehrish et al. 2023).

Microgreens can be dehydrated by available techniques. Blanching prior to drying will deactivate the enzyme activity and will be helpful in retaining more color. After dehydrating can be incorporated in different ready-to-eat food adjuncts like chutney powders and soup mixes which will enable the benefits of microgreens for a prolonged duration. Klopsch et al. (2018) noted that incorporating pea and lupin microgreens into bread increased the levels of health-promoting secondary plant metabolites such as kaempferol, quercetin derivatives, and genistein in the bread. This suggests that bread can be customized to meet specific consumer health requirements using these natural components. Kaur et al. (2022) used wheatgrass (WP) and mung bean (MP) powder to prepare gluten-free eggless muffins. Both powders were added to the rice flour at 2%, 4% and 6% concentrations. Viscoelasticity of the muffin batter increased with increasing rate of WP and MP powders, along with the gumminess, hardness, and chewiness of muffins. This, however, went hand in hand with reduced muffin height and specific volume. MP

incorporated muffins also exhibited elevated concentrations of free and bound flavonoids while WP incorporated muffins showed elevated concentrations of free and bound phenolics. Both WP and MP muffins also had increased concentration of proteins, dietary fibres, and antioxidant activities. In terms of sensory qualities, 6% MP muffins scored lower than the rest while 2% WP were evaluated to be the most acceptable in terms of sensory characteristics.

Devi et al. (2023) prepared instant chutney powders from fenugreek microgreens. Ingredients used were microgreen powder (5–25%), black gram dhal, bengal gram dhal, cumin and coriander seeds, garlic, tamarind, chilli powder and common salt. The developed chutney was having high organoleptic acceptability, however, the sensory scores decreased with increasing concentrations of microgreen powder. Similarly, Sravishta et al. (2023) developed acceptable qualities of novel value-added products like ready-to-serve instant soup mix, instant chutney powders and sauces, and health mix using fenugreek and horse gram microgreen powders. Frozen Kebab were developed by incorporating 60% soya kheema, 20% germinated brown rice, 10% fenugreek microgreens and 10% mustard microgreens (Munde et al. 2023). The developed product was having high organoleptic acceptability and contained sufficient amount of nutrients such as protein, carbohydrates, crude fiber, dietary fibers (12.66%, 26.64%, 7.41% and 2.79%, respectively). Kumari et al. (2023) described the recipes for preparation of several Indian dishes like *pulav*, *utthapa*, *idli*, *potato tikki*, *poha*, etc. with various microgreens. All the items had exceptional nutritional qualities, while their flavor was deemed satisfactory. However, they slightly lagged in terms of look and texture.

7 Future Trends

Because microgreens have a short growth cycle, they can be cultivated with minimal effort. They can even be grown at home without the use of pesticides and collected as needed. As a result, they have a small impact on the environment and are widely accepted by health-conscious consumers. The influence of ambient and stimulating variables on the nutritional profile and phytochemical concentration in microgreens is crucial. The components encompassed in this context are the growth environment, cultivation substrates, induced environmental stresses, seed priming and biostimulants, biofortification, and the impact of light in controlled conditions. An in-depth analysis of the microgreen market trends is also necessary. One can also investigate and employ integrated multiomics methodologies, together with nutriomics and foodomics, to discover and cultivate microgreen genotypes with the greatest potential for rapid growth, excellent nutritional values, high germination rates, extended shelf-life, and suitability for food consumption. The microgreen diet tailored to the body's need can improve physical health by providing the necessary nutrients for optimal function, reducing the risk of nutrient deficiencies, and managing health conditions effectively. The potential of microgreens as an innovative and environmentally friendly food source is thoroughly exploited through research on food-based analytical variables, which empowers both the producers and consumers. It also serves as a foundation for further research.

Underutilised plants, wild food plants, or crops' wild relatives, which are frequently preserved in genebanks, have the potential to provide great chances for producing microgreens that are both highly nutritious and have unique and appealing flavours. This would cater to the needs of health-conscious consumers. Additional efforts are essential to enhance both production and storage conditions in order to boost the safety, quality, and shelf

life of microgreens, thus broadening the scope of possible markets. It is essential to optimize MAP for various types of microgreens. Using unsuitable modified atmospheres might cause physiological diseases, hinder wound healing, accelerate ageing, and make microgreens more vulnerable to pathogen growth and decay. Both a well-maintained cold chain and appropriate MAP are crucial to minimize microgreens respiration, prevent moisture loss, reduce environmental contamination, and inhibit the growth of microbes causing spoilage and diseases. These measures effectively extend their shelf life.

Utilizing genetic biodiversity is necessary to acquire customized microgreens that possess specific nutritional profiles, especially in terms of mineral elements and bioactive chemicals. It is important to create suitable procedures before and after harvesting microgreens in order to maximize their retention of nutritional content. While it is important to extend their shelf life, a potential area to conduct future research is exploring innovative methods of processing and reformulating discarded microgreens into new products. In addition, as microgreens are often eaten raw, there is minimal loss or deterioration of heat-sensitive micronutrients or vitamins when ingested in their fresh state. However, when processed into various value-added products, novel processing techniques should be explored to preserve their nutritional status. Further research is needed to comprehend the distinct and combined health impacts of microgreens. Additional clinical and experimental trials are also required for complete understanding of efficacy of microgreens on the human body.

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Microgreens as Budding Medicine and Bountiful Source of Nutrition

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Abbreviations

B2B Business to Business
B2C Business to Customer
GC/MS Gas Chromatography-Mass Spectrometry
DPPH 2-diphenyl-1-picryl-hydrazy
WHO World Health Organization
CAGR compound annual growth rate
HoReCa Hotel Restaurant and Catering

1 Introduction

The popularity of microgreens has surged in recent times due to their nutritional value, health advantages, and ease of cultivation. Microgreens are tiny seedlings of various vegetables and medicinal plants which are harvested at a tender age of just 2–3 weeks after germination. They serve as a nutritious addition to daily diets, supplements, and other culinary activities. Unlike their mature counterparts, microgreens offer enhanced nutritional profiles without compromising taste, making them a preferred choice for health-conscious individuals and chefs alike. People have been consuming immature vegetable plants since ages but the trend of microgreen began in 1980s at fancy-restaurants for garnishing the dishes

and later became popular as functional food (Verlinden 2020). Microgreens are becoming more and more well-liked due to their culinary diversity and health advantages, which presents a profitable business prospect. This is a comprehensive guide on marketing tactics for a microgreens company. Microgreens are a broad range of vegetables, herbs, and medicinal plants, each with its own distinct tastes and nutritional advantages. They are the seedlings of edible plants that are picked 7–14 days after sowing when the first genuine leaves appear (Turner et al. 2020). Arugula, basil, beet, broccoli, cabbage, cilantro, mustard, and radish microgreens are among the common variations; there are also specialist species like amaranth and pea shoots (Turner et al. 2020) (Fig. 1 and Table 1).

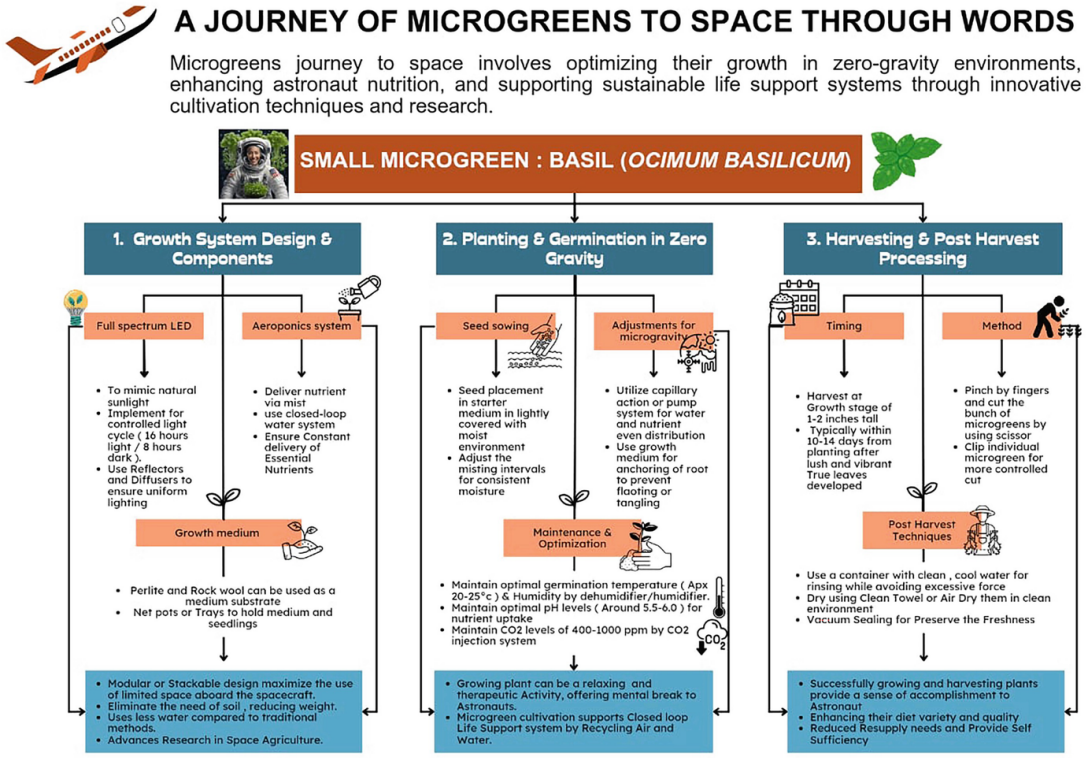


Fig. 1 A Journey of Microgreens to Space: This diagram outlines the cultivation of basil microgreens in space, detailing the growth system design, planting and germination in zero gravity, and harvesting processes to optimize astronaut nutrition and sustainable life support systems

Table 1 Brief description of diverse microgreens and its growth conditions with nutrient value

S. No	Crop species	Family	Days toHarvest	Growing conditions (Best suited)	Nutritional Highlights	References
Vegetables						
1.	Broccoli (*Brassica oleracea* var. *italica* Plenck) and Radish	Brassicaceae	Broccoli- 13 Radish- 10	Hydroponic medium at temperature 25 °C and 16/8 h of photoperiod	No notable differences in glucosinolates and total phenolics level. Reduced	Ortiz et al. (2024)

S. No	Crop species	Family	Days toHarvest	Growing conditions (Best suited)	Nutritional Highlights	References
	(*Rhaphanus sativus* L.)				anthocyanin for radish when harvested late.	
2.	Arugula (*Eruca sativa* L.)	Brassicaceae	14	Hydroponic culture at 25 °C, with seeds soaked for 48 hours, placed in phenolic foams, irrigated with a nutrient solution, and kept in a controlled environment, with a 12-hour photoperiod	Protein content: 18.2%. Total fiber content: 28.7%. Soluble fiber content: 6.7%. Zeaxanthin and β-carotene are the predominant carotenoids, while flavonoid-derived O-glucosides are also present.	Komeroski et al. (2023)
3.	Beetroot (*Beta vulgaris subsp. Vulgaris*), Palak (*Beta vulgaris var. bengalensis*), Amaranthus (*Amaranthus viridis*)	Amaranthaceae	Beetroot- 10 Palak- 10 Amaranthus- 9	Coco peat medium	Beetroot: High in carotenoids, P, K, Ca and Mg but low in ascorbic acid. Palak: High in Ca, Fe, K and Ascorbic acid but low in P and Mg. Amaranthus: High in N, K, Ca Fe and ascorbic acid but low in P, Mg and Zn	Ramya et al. (2022)
4.	Lettuce (*Lactuca sativa)*	Compositae	9	Coco peat medium	High in N, Zn, ascorbic acid and moisture content but low in carotenoids.	Ramya et al. (2022)
Cereals						
5.	Wheat (*Triticum aestivum*)	Poaceae	8	Coco peat medium	High in N, K and ascorbic acid but low in Ca and Fe.	Ramya et al. (2022)
6.	Wheat Landraces: (*Triticum*	Poaceae	Wheat: 7 Barley: 9	Combination mixture of sterile peat, coconut shell,	Wheat landraces: High in Total	Altuner et al. (2021)

S. No	Crop species	Family	Days toHarvest	Growing conditions (Best suited)	Nutritional Highlights	References
	aestivum L. and *T. aestivum*), Wheat cultivar (*T. aestivum L. emend. Fiori et Paol.*), Barley (*Hordeum vulgare* L.) and Oats (*Avena sativa L. and A. sativa*)		Oats: 8 and 13	and perlite at 21 ± 2 °C/17 ± 2 °C of temperature, 50–60% of humidity with 16/8 hour of photoperiod.	anti-oxidant capacity (TAC) and Total phenolic content (TPC). Wheat cultivar: High in anthocyanin content. Barley displayed elevated N balance index. Oats: High in ascorbic acid and flavonol contents.	
Legumes						
7.	Fenugreek (*Trigonella foenum-graecum L.*), Mung bean (*Vigna radiata (L.) R. Wilczek*), Cowpea (*Vigna unguiculata (L.) Walp*) and Horse gram (*Macrotyloma uniflorum (Lam.) Verdc*)	Fabaceae	8–10	Soil and coco peat medium. From nutritional perspective, soil medium was the best whereas from growth perspective, coco peat medium outperformed.	When compared to sprouts, microgreens have elevated carotenoids, ascorbic acid, chlorophyll, display more anti-diabetic and anticholinergic properties and have minerals like Zn, Fe, Cu, etc.	Eswaranpillai et al. (2023)
8.	Fenugrek (*Trigonella foenum-graecum),* Green gram (*Vigna radiata L.*) and Peas (*Pisum sativum*)	Fabaceae	Fenugreek: 11 Peas: 7 Green gram: 7	Coco peat medium	Fenugreek: High in iron and ascorbic acid Green gram: High in N and low in K, Ca, Fe and chlorophyll. Peas: High in N, K, ascorbic acid and carotenoids.	Ramya et al. (2022)
9.	Mungbean (Vigna radiata L.) and Lentil	Fabaceae	7	Distilled water was used to soak the seeds for 24 hours and were sown homogenously on	Both Mungbean and Lentil displayed an elevated	Dhaka et al. (2023)

S. No	Crop species	Family	Days toHarvest	Growing conditions (Best suited)	Nutritional Highlights	References
	(Lens culinaris L.)			the trays filled with the sand, vermiculite, and coco peat mixture, under 20 Watt of LED light for 16/8 hours of photoperiod at temperature 20–30 °C with 65–75% humidity.	nutritional profile including antioxidant, vitamins and dietary fibre.	
Oilseeds						
10.	Nigella (*Nigella sativa L.*), Safflower (*Carthamus tinctorius L.*), and Camelina (*Camelina sativa L.*)	Ranunculaceae, Asteraceae, and Brassicaceae, respectively.	Camelina and Safflower: 11 days after sowing, while Nigella was harvested after 24 days (1st harvest). Camelina and Safflower: 18 days after sowing, while Nigella was harvested after 30 days (2nd harvest).	Light peat and water soluble fertilizer were used as a growing medium in seed boxes, with a pH of 6. The greenhouse had a temperature of 18–21 °C with humidity ranging from 50 to 88%.	Oilseeds microgreens at 1st harvest demonstrated high concentrations of nutrients like fat, protein, K, P, Se, Fe (excluding safflower) and Ni comparatively. It was found that the levels of dietary fibre, carotenoids, polyphenols and Vitamin C increased as the microgreens grew old.	Kapusta-Duch et al. (2024)
Herbs						
11.	Anise (*Pimpinella anisum L.*), Chervil (*Anthriscus cerefolium L.*), Caraway (*Carum carvi L.*), and Dill (*Anethum graveolens L.*)	Apiaceae	Anise, Chervil, and dill: 20 days and Caraway at 23 days	Peat-based medium was used and microgreens were grown in controlled environment.	Anise outperformed others in terms of yield and lutein content whereas Chervil and Dill showed the highest level of ascorbic acid. All four herbs displayed an abundance in polyphenols, flavonoids and	Giordano et al. (2022)

S. No	Crop species	Family	Days toHarvest	Growing conditions (Best suited)	Nutritional Highlights	References
					phenolic acid along with Caraway and Dill exhibiting a strong antioxidant activity.	

Because microgreens are fragile, extending their shelf life is essential to successful marketing. When kept at 5 °C, fresh-cut microgreens can last up to 10 days on an average; Nevertheless, they are susceptible to wilting, dehydration, and loss of nutrients (Yan et al. 2022). It is crucial to use the right pre-harvest and postharvest treatments in order to prolong their freshness. This entails utilizing gentle handling techniques, maximizing growth conditions, and making sure that proper moisture management is maintained throughout packing and storage. Their quality can also be maintained by packaging them in macro-perforated packaging that keep out moisture while maintaining humidity (Ghoora et al. 2020).

Furthermore, teaching customers about appropriate home storage techniques may extend the shelf life of microgreens, guaranteeing that they stay attractive and fresh from farm to table. It is crucial to raise product awareness before releasing a product onto the market if consumers don't know about it. The public can be given testers or informed on the nutritional value to accomplish this (Van Rooyen and Eindar 2021). These precautions assist microgreens' marketability and customer pleasure in addition to preserving their nutritional content and visual attractiveness.

2 Microgreens- the Instant Functional Super Food

Functional foods are foods that not only provide essential nutrition, but also contain additional components such as bioactive compounds or phytochemicals. Presence of these can enhance certain aspects of health, such as immunity, inflammation, digestion and even risk of certain life-threatening ailments like cancer, diabetes, hypertension and cardiovascular diseases. The concept of functional foods originated in Japan in the 1980s, but it is not legally recognized or standardized in most other countries (Granato et al. 2020). According to a latest definition by Granato et al. (2017) functional foods are either natural or artificially processed foods whose inclusion in routine diet at efficient levels may help in improving health. One example of functional foods is microgreens, which are young plants that are harvested before they develop true leaves. They are petite greens, comprising nutrient-dense leaves produced from various groups of plants such as legumes, cereals, vegetables and oil-seeds, consumed as vegetables alongside fragile petioles and shoots to complete a balanced

meal. They are abundant in nutritional fiber and can be eaten raw or cooked. However, their nutritional values vary from crop to crop at the same stage and the conditions provided at the time of growth (Nair 2023).

They gained popularity in Southern California in the 1990s and were dubbed as “Functional food” or “Superfoods” because of their superior nutritional and antioxidant value (Gupta et al. 2023). Microgreens are abundant in many different minerals (iron, calcium, magnesium) and plant chemicals, including lutein, vitamin C, beta-carotene, vitamin E, carotenoids, flavonoids, vitamin K, and polyphenols, that improve human health (Zhang et al. 2021). By regulating oxidative stress, inflammation, and metabolic pathways, these substances can help prevent or treat chronic illnesses like diabetes, cancer, neurological conditions, cardiovascular and immunological diseases (García-Tenesaca et al. 2024).

2.1 Microgreens as Medicine

Since microgreens are the cotyledons of various pulses, grains, and vegetable seeds, their nutritional profile differs from that of the whole plant. Microgreens of these plant includes essential fatty acids, of which linoleic acid is the main one, as well as functional compounds and important antioxidant enzymes like glucoraphanin, which signals the antioxidants, glucosinolates, isothiocyanates, anthocyanins, ascorbate peroxidase, catalase, superoxide dismutase, and peroxidase. These compounds act against oxidative stress and inflammation, and as a result, they protect against chronic diseases like hypertension, cardiovascular disease, diabetes, and cancer (Sehrish et al. 2023).

Microgreens are prized for their powerful therapeutic qualities as well as their culinary applications. Broccoli, radish, cilantro, and pea shoots are among the varieties that stand out for having high levels of vitamins, minerals, and antioxidants. Broccoli microgreens, are high in sulforaphane (Kathi et al. 2023) a substance with anti-cancer potential, while cilantro microgreens are high in vitamins A, C, and K as well as having detoxifying characteristics.

It was discovered that the microgreens provided an moderate to extreme source of dietary fiber, protein, and vital components. They fulfilled 2816%, 2832%, and 2472% of the recommended daily intake of ascorbic acid, vitamin E, and beta-carotene (pro-vitamin A) respectively, making them good providers of these nutrients (Ghoora et al. 2020). Microgreens may be tiny, but their bioactive components offer significant nutraceutical advantages, encouraging long-term health maintenance through frequent ingestion. Through Se biofortification, microgreens’ nutraceutical value may be further increased, providing meals that are richer in Se and may have a higher concentration of bioactive components (Pannico et al. 2020). The best way to sell these microgreens is to highlight their exceptional nutritional value and therapeutic qualities, showing them as vital parts of a well-rounded, health-conscious diet.

2.2 Shelf Life of Medicinal Microgreens

Medicinal microgreens have a short shelf life; After harvesting, microgreens has been stored at 10 °C for 7–14 days, and under refrigeration, the shelf life increases to 12–14 days at 4 °C (Sharma et al. 2023). Because of their fragility, they are prone to wilting, dehydration, and nutritional loss. Using efficient management techniques is essential to extending their shelf life. This entails using careful handling practices when harvesting and maximizing growth conditions with enough light, water, and nutrients. Microgreens must be stored in permeable containers that keep moisture in while avoiding condensation after harvest in order to preserve their nutritional value and freshness. To reduce harm to the fragile tissues during harvest, gentle methods should be used.

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3 Medicinal Properties of Microgreens

Cruciferous microgreens' rich nutrient and phytochemical content is making their possible anti-cancer effects better known. These immature seedlings are incredibly nutrient-dense, having been picked only a few weeks after germination.

Diets high in cruciferous vegetables, according to the World Cancer Research Fund, are especially protective against thyroid, colon, and rectum cancers. Important constituents like polyphenols and other phytochemicals are essential to their anti-cancer properties. Moreover, several antioxidant bioactive substances linked to cruciferous vegetables, including ascorbic acid, tocopherols, carotenoids, polyphenols, and glucosinolates, have been implicated in the protection of chronic illnesses (de la Fuente et al. 2020). In this way, eating Brassica greens has been linked to a lower chance of developing cancers of the stomach, pancreas, colon, lung, breast, and ovary.

Microgreens are essential for the body's immune system's ability to fight cancer since studies have demonstrated that they can reduce free radicals, cause cancer cells to undergo apoptosis, and boost the activity of natural killer cells. In particular, sulforaphane, a substance believed to help inhibit cancer cell development and proliferation (Kamal et al. 2020) is abundant (10–100 times) in microgreens like broccoli (Triska et al. 2021). Ascorbic acid, α-tocopherol, vitamin E, and beta-carotene are among the antioxidants found in broccoli sprouts that may help prevent cardiovascular illnesses (Aziz et al. 2022).

Furthermore, the high antioxidant content of microgreens aids in the reduction of oxidative stress, a major contributor to the development of cancer. According to research, eating these nutrient-dense plants on a daily basis can improve general health and work as a cancer prevention strategy.

3.1 Coriander as an Anti-Diabetic

Coriandrum sativum (Coriander) belongs to Apiaceae family. Coriander leaves are very well known for their numerous benefits on human health.

When coriander microgreens were analyzed using gas chromatography-mass spectrometry (GC/MS), bioactive substances like phenolic amide, imidazo pyridazine, thienopyrimidines, phenolic components, and essential oil were found. Stems, terpenoids, essential oils, phenolic chemicals, and fatty acid esters were abundant in mature leaves. These compounds are all recognized for their anticarcinogenic, antidiabetic, and therapeutic antioxidant qualities (Dhakshyani and Alias 2022).

3.2 Health Benefits of Chickpea Microgreens

Like other microgreens, Chickpea (*Cicer arietinum L.*) microgreens are rich in nutrients and bioactive substances that have a host of health advantages. These young plants are very high in vitamins (Ebert 2022), minerals, and dietary fiber, all of which support general health and aid in the treatment of a number of illnesses. Compared to raw seeds or sprouts, chickpea microgreens were higher in vitamins and antioxidant activity (Kurian and Megha 2020; Kumar et al. 2023). Chickpea microgreens' high fiber content eases constipation and encourages a healthy digestive tract, both of which are beneficial to gut health.

3.3 Microgreens as Nutraceutical

The world's population requires novel, readily consumable food sources with unique nutraceutical qualities in the twenty-first century. Microgreens may be considered one of the primary protagonists of this impending transformation in this particular situation (Marchioni et al. 2021).

Broccoli seedlings (*Brassica oleracea L. var. Italica*) are highly valued due to its significant bioactive content, which includes vitamins, vital minerals, phenolic compounds, and glucosinolates. There is a favorable correlation between these secondary metabolites and possible health advantages. The biological characteristics of broccoli seedlings include anti-inflammatory, anti-cancer, antibacterial, antioxidant, and antidiabetic effects. Therefore, since these foods may offer basic nutrition as well as health advantages to lower the risk of chronic illnesses, functional foods and nutraceuticals are receiving a lot of attention (Le et al. 2020).

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4 Micro-to- Macro Nutrient Composition of Microgreens

Micronutrients are those essential elements which are needed by plants in a minute proportion whereas macronutrients are required in comparatively higher quantity. Meanwhile macronutrients provide energy (calories), micronutrients are essential for supporting metabolic processes, immune function, and overall health, even though they are required in smaller quantities. A balanced diet that includes a variety of nutrient-dense foods is essential for meeting both macro and micronutrient needs.

4.1 Micronutrients

Microgreens are a remarkable source of many vitamins and minerals, each of which has a distinct effect on general health and wellness (Feng et al. 2022).Vitamin C, prominent for its immune-boosting qualities and its function in collagen production, which promotes skin health and wound healing. Vitamin C is found in abundance in microgreens like broccoli, kale, green perilla, and red cabbage (Bhaswant et al. 2024; Kathi et al. 2023). Microgreens made from spinach and swiss chard are high in vitamin K, which is essential for bone metabolism and blood clotting. Sufficient consumption of vitamin K promotes bone density and may lower the chance of fractures. High concentrations of vitamin E, a powerful antioxidant that shields cells from oxidative damage and boosts immunity, are found in microgreens like sunflower and pea shoots.

Beta-carotene, a precursor to vitamin A, is abundant in carrots and beet green microgreens. This vitamin is necessary for healthy skin, immunological system, and vision.

The several vitamins B included in microgreens are essential for cellular health and energy metabolism. The different quantities of thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6), and folate (B9) all support general health and vitality. Notable sources of iron include spinach and beet green microgreens, which are essential for the blood's oxygen delivery process. However, an iron deficit may cause weakened immunity and weariness.

Rich in calcium, broccoli and kale microgreens are necessary for healthy bones and muscles. Sufficient consumption of calcium is essential for averting osteoporosis and preserving optimal neurological performance. Potassium is essential for blood pressure regulation, fluid balance, and heart function. Swiss chard and arugula microgreens are good sources of this mineral.

Good sources of magnesium include microgreens like basil and Swiss chard. Magnesium is necessary for healthy bones, blood sugar management, and muscle and nerve function. Zinc found in sunflower and radish microgreens, is necessary for DNA synthesis, wound healing, and immune system activity. In addition to helping to maintain healthy skin, zinc may lessen the intensity and length of colds.

4.2 Macronutrients

Although microgreens are mainly appreciated for their micronutrient content, they also make a minor but significant contribution to the consumption of macronutrients when compared to other dietary sources. *USDA Food Data Central. (Accessed January 2024).*The amounts of proteins in microgreens varies, with certain types having a higher protein content than others (Mathew et al. 2022). For instance, pea shoots are well-known for having a higher protein content than other microgreens. Even while they might not contain as much protein as meat or legumes, microgreens can

nevertheless add to a person's daily protein consumption, especially for people who eat a plant-based diet. Carbohydrates are found in microgreens as sugars and dietary fiber. Plant cell walls include dietary fiber, which is essential for maintaining a healthy digestive system, encouraging regular bowel movements, and helping with weight management by increasing satiety. Microgreens usually have a low sugar content, mostly simple sugars that give the body instant energy.Although microgreens typically don't contain much fat, some types, including flax microgreens, may have trace levels of healthful fats, such as omega-3 fatty acids. These fats are essential for heart and brain function as well as for lowering inflammation. Even though microgreens have less fat than other food sources, eating a range of microgreens can increase total fat consumption and supply important fatty acids that are needed for good health.

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5 Leguminous Microgreens

Made from the seeds of plants that are considered to be legumes, leguminous microgreens provide an abundance of nutritional advantages, especially for protein and other vital elements. Let's examine the nutritional makeup and health advantages of leguminous microgreens in more detail:

Leguminous microgreens, such as lentil microgreens and pea shoots, are well known for having a high protein content. These microgreens are an excellent source of plant-based protein for vegetarians, vegans, and anyone trying to cut back on meat because they can have up to 30% protein by dry weight. Since leguminous microgreens contain all nine essential amino acids required for human health, their protein is regarded as complete. Because of this, legume microgreens are a great substitute for animal products as a source of protein.

5.1 Nutrition Composition and Importance of Diverse Legume Crops

5.1.1 Dietary Fiber

Leguminous microgreens include dietary fiber, both soluble and insoluble, in addition to protein. Dietary fiber is essential for maintaining a healthy digestive system because it encourages regular bowel movements, avoids constipation, and helps people feel fuller for longer, which helps with weight management (Feng et al. 2022). Leguminous microgreens include soluble fiber, which lowers cholesterol, controls blood sugar, and enhances insulin sensitivity. By giving the stool more volume, insoluble fiber helps waste move through the digestive system more easily and lowers the risk of colon cancer.

5.1.2 Beneficial Fats

Although leguminous microgreens don't usually have a lot of fat, they might contain trace levels of omega-3 fatty acids and other beneficial fats. Alpha-

linolenic acid (ALA), in particular, is an omega-3 fatty acid that is critical for brain function, cardiovascular health, and the reduction of inflammation in the body.

Leguminous microgreens are a good way to boost consumption of omega-3 fatty acids, especially for plant-based diet followers who might not have access to other sources of these important fats.

5.1.3 Rich Nutrient Content

As microgreens are miniature, young plants harvested at an early stage of growth, and they are renowned for their exceptional nutrient density. The inclusion of microgreens in diets can be particularly beneficial due to their high content of essential nutrients, including calcium and magnesium. Microgreens, such as those from the Brassica family, are rich sources of vitamin C and minerals such as copper and zinc. (Zhang et al. 2021) Microgreens boast an array of phytochemicals, carotenoids, and phenolic compounds. These bioactive compounds act as antioxidants, helping combat oxidative stress and inflammation in the body. Vitamin E, present in microgreens, contributes to improved body functioning. Microgreens, with their high fibre content and vitamin K levels, can contribute to heart health. Foods rich in fibre and vitamin K, like microgreens, may assist in maintaining healthy blood pressure and reducing the risk of heart-related issues (Eliseeva and Simina 2021). Emphasizing the substantial amount of essential nutrients, including calcium and magnesium, found in microgreens. These nutrients are vital for bone health, muscle function, and metabolic processes (Zhang et al. 2021). Microgreens, particularly from red Salanova cultivars, are high in essential nutrients such as calcium and magnesium, contributing to their potential as nutrient-dense food sources (Gupta et al. 2023). Microgreens of lentil and mung bean were grown in mixture of cocopeat (1.5): vermiculite (1.5): sand (1) under warm LED lamps and 16/8 hours light/dark photoperiod (Dhaka et al. 2023). These microgreens exhibited 81% and 80% antioxidant activity (measured as DPPH scavenging potential), concentration of total phenols—988.24 and 840.68 (µg GAE/100 g FW), ascorbic acid content—16.34 and 48.56 (mg/100 g FW) respectively.

5.1.4 Protein and Fibre Sources

Microgreens, across various cultivars, are moderate to good sources of protein and dietary Fibre, emphasizing their nutritional significance. Microgreens are emerging as noteworthy sources of both protein and dietary Fibre, showcasing their nutritional prowess across different cultivars. These miniature versions of mature vegetables are packed with essential nutrients, making them an excellent addition to the diet (Ebert et al. 2022). Microgreens contain moderate to substantial amounts of protein, contributing to their nutritional value. While protein levels may vary among different varieties, they offer a notable source for those seeking plant-based

protein alternatives (Renna and Paradiso 2020). Similarly, microgreens exhibit significant dietary fibre content, which is crucial for digestive health and overall well-being. Their fibre content varies across cultivars but remains consistently noteworthy, promoting satiety and aiding in digestion (Renna and Paradiso 2020). This dual benefit of providing both protein and Fibre underscores the importance of incorporating microgreens into one's diet. Whether used in salads, smoothies, or as garnishes, microgreens offer a convenient and nutritious way to enhance meals and promote health.

5.1.5 Variety in Nutrient Profile

Microgreens, including lettuce landraces, exhibit diverse nutrient profiles, making them appealing to consumers seeking high-quality products. Microgreens, encompassing lettuce landraces among others, showcase a rich tapestry of nutrient profiles, amplifying their allure for health-conscious consumers. These miniature greens offer a diverse array of essential vitamins, minerals, and phytonutrients, reflecting their varied genetic backgrounds and cultivation methods (Martínez-Ispizua et al. 2022). Microgreens are abundant in vitamins such as vitamin C, vitamin K, and various B vitamins, depending on the specific variety. For example, green daikon radish microgreens are rich in vitamin E, while others like broccoli microgreens exhibit high concentrations of sulforaphane, known for its anti-cancer properties (Zhang et al. 2021). They are also a good source of essential minerals like potassium, phosphorus, calcium, zinc, iron, magnesium, and sodium (Bhaswant et al. 2023). Different varieties may have varying mineral concentrations, contributing to their nutritional diversity and appeal. Microgreens contain a wide range of phytonutrients, including antioxidants and other bioactive compounds, which contribute to their health benefits (Zhang et al. 2021). These compounds help combat oxidative stress, reduce inflammation, and support overall health and well-being.

5.1.6 Microgreens from Medicinal Herbs and Leguminous Crops

Legumes with small seeds, including red clover and alfalfa, are a great source of nutrients and bioactive substances for all phases of growth, from seeds to sprouts to microgreens. These legumes are prized for their bioactive chemicals, such as flavonoids and phenolic acids, which have anti-inflammatory and antioxidant qualities, in addition to their high protein content and other necessary minerals (Chiriac et al. 2020).

Furthermore, the genetic variety of these crops presents prospects for agricultural development initiatives that seek to augment their nutritional value and resistance.

A range of leguminous plants provide the vitamins, minerals, and other vital resources that legume microgreens need to nourish themselves (Wang

et al. 2021). The list of common nutritious leguminous plants has been discussed below and depicted in Table 2.

Table 2 Mineral analysis for mung bean, lentil, green pea and alfalfa

S.No	Legume	Mineral	Quantity	References
1.	Mung bean	Potassium (K)	239–369 mg/100 g	Priti et al. (2021)
		Phosphorus (P)	66–87 mg/ 100 g	
		Calcium (Ca)	47–87 mg/100 g	
		Magnesium (Mg)	36–56 mg/100 g	
		Sodium (Na)	30–60 mg/100 g	
2.	Lentil	Potassium (K)	299–389 mg/100 g	Priti et al. (2021)
		Phosphorus (P)	32–46 mg/100 g	
		Calcium (Ca)	45–70 mg/100 g	
		Magnesium (Mg)	29–66 mg/100 g	
		Sodium (Na)	27–45 mg/100 g	
3.	Green Pea	Potassium (K)	23.2 g (kg^{-1} DW)	García-Tenesaca et al. (2024)
		Phosphorus (P)	7.7 g (kg^{-1} DW)	
		Calcium (Ca)	4.8 g (kg^{-1} DW)	
		Sulphur (S)	6.4 g (kg^{-1} DW)	
		Magnesium (Mg)	3.1 g (kg^{-1} DW)	
4.	Alfalfa	Potassium (K)	15 g (kg^{-1} DW)	García-Tenesaca et al. (2024)
		Phosphorus (P)	6.2 g (kg^{-1} DW)	
		Calcium (Ca)	6.8 g (kg^{-1} DW)	
		Magnesium (Mg)	4.2 g (kg^{-1} DW)	

5.1.6.1 Peas

As a valuable source of plant-based protein in leguminous microgreens, peas have a high protein content. Both soluble and insoluble fiber are included in peas, which support healthy digestion and help control weight. Vitamins C, K, and B vitamins, especially folate (B9), which is necessary for cell division and DNA synthesis, are all present in peas. Minerals such as iron, calcium, potassium, and zinc are abundant in peas and are essential for a number of body processes, including immune system support, bone health, and electrolyte balance (Barsila et al. 2023).

5.1.6.2 Lentils

Lentils are well known for having a high protein content. They provide vital amino acids that are required for cellular activity and muscle repair (Hang et al. 2022). High in dietary fiber, lentils support healthy digestion, control blood sugar, and decrease cholesterol. Folate, which is necessary for foetal development during pregnancy, and vitamin B6, which is important in

neurotransmitter synthesis, are both found in lentils. Minerals like Iron, magnesium, and potassium are among the minerals found in lentils that are essential for heart health, muscle function, and the metabolism of energy.

5.1.6.3 Chickpeas

Leguminous microgreens have a significant quantity of plant-based protein, which is abundant in chickpeas (Mathew et al. 2022). Soluble and insoluble fiber found in chickpeas aid in digestion, increase satiety, and improve heart health. High in vitamin B6, which is essential for brain function and metabolism, and vitamin K, which is necessary for blood clotting, are found in chickpeas. Iron, manganese, and phosphorus are among the minerals found in chickpeas that are important for the synthesis of red blood cells, the health of bones, and the metabolism of energy.

5.1.6.4 Soybeans

Packed full of all the essential amino acids, soybeans are one of the few plant-based foods that meet this requirement. Dietary fiber, which is abundant in soybeans, promotes healthy digestion and aids in blood sugar regulation. Vitamin B6, which is essential in metabolism and brain function, and vitamin K, which is crucial for bone health, are both abundant in soybeans (Kim et al. 2024). Calcium, magnesium, and potassium are among the minerals found in soybeans and are crucial for maintaining electrolyte balance, muscle strength, and bone health.

5.1.7 Phytochemicals

Flavonoids, phenolic compounds, and antioxidants are among the phytochemicals found in legume microgreens. These compounds have a number of health-promoting qualities, such as anti-inflammatory and antioxidant activities (Feng et al. 2022). Leguminous microgreens, which come from legumes such as chickpeas, peas, and soybeans, can be a rich source of vital nutrients that promote general health and wellbeing when incorporated into a diet. These nutrient-dense microgreens provide an easy and long-lasting solution to increase nutrient diversity and encourage a well-balanced diet (Wang et al. 2021).

5.1.8 Medicinal Microgreens and Its Nutritional Content

These microgreens are prized for their powerful therapeutic qualities as well as their culinary applications. Broccoli, radish, cilantro, and pea shoots are among the varieties that stand out for having high levels of vitamins, minerals, and antioxidants. Broccoli microgreens, for example, broccoli are high in sulforaphane (Kathi et al. 2023) a substance with anti-cancer potential, while cilantro microgreens are high in vitamins A, C, and K as well as having detoxifying characteristics. In addition, these microgreens provide a wealth of vital minerals that support general health and wellness, including potassium, iron, and magnesium. Their high polyphenol content increases their antioxidant potential even more, promoting cardiovascular

health and helping to regulate plasma lipoprotein. Microgreens may be tiny, but their bioactive components offer significant nutraceutical advantages, encouraging long-term health maintenance through frequent ingestion. The best way to sell these microgreens is to highlight their exceptional nutritional value and therapeutic qualities, showing them as vital parts of a well-rounded, health-conscious diet.

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6 Marketing Strategies of Microgreens: Laboratory to Market (Farm to Fork)

6.1 Determine the Target Market

In this light, the Knowledge Sourcing Intelligence (2020) indicated that the global microgreens market size reached US$ 1522 million in 2022 and is expected to reach US$ 2470 million by 2028, exhibiting a compound annual growth rate (CAGR) of 8.4% during 2022–2028 (Flores et al. 2024). Determining the intended audience is essential for every kind of business, including microgreens businesses. This entails being aware of the requirements, tastes, and demographics of potential clients. The target market for microgreens usually consists of local markets and restaurants seeking premium ingredients to elevate their recipes, as well as health-conscious consumers wanting wholesome and fresh food (Van Rooyen and Eindar 2021). Restaurants frequently hunt for microgreens to enhance the flavor and presentation of their food in order to satisfy patrons who are searching for novel and healthful choices.

Microgreens are popular among health-conscious customers because of their high vitamin content and adaptability in the kitchen—they work well in salads, smoothies, and garnishes (Enssle 2020). For those who want to buy fresh goods and support local farmers, local markets offer a direct sales channel. Through the identification and comprehension of these target categories, microgreens enterprises may customize their marketing strategies and product offerings to efficiently cater to the demands and preferences of their clientele, hence propelling sales and business expansion (Figs. 2 and 3).

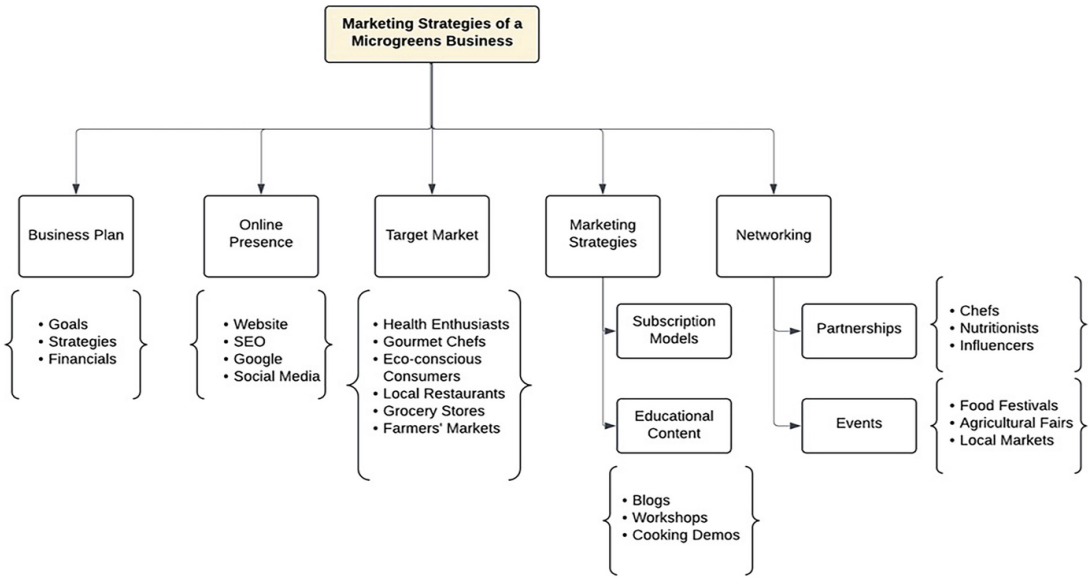


Fig. 2 Marketing strategies of microgreens for business purpose

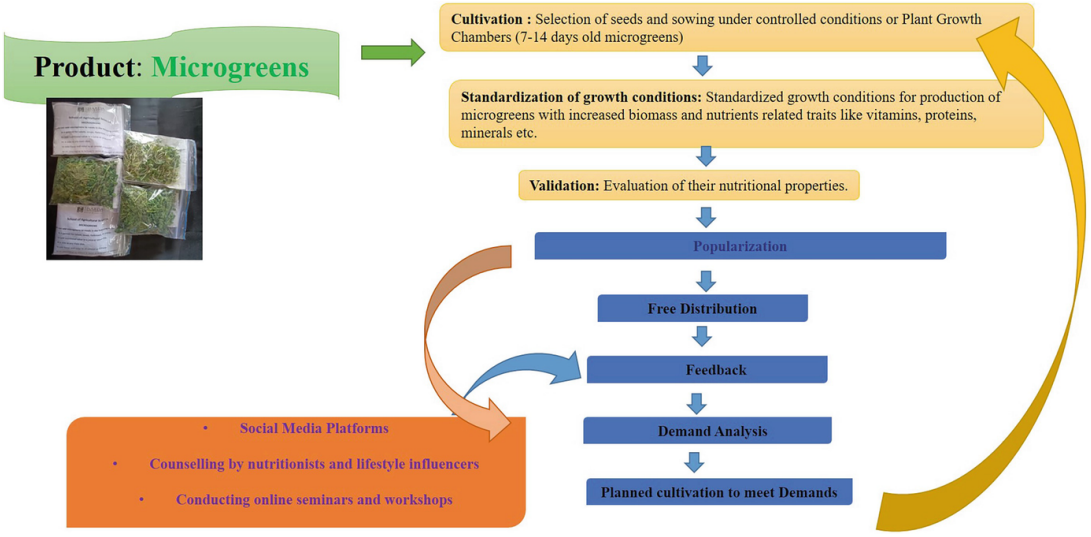


Fig. 3 Business model fine points for microgreens advertising

6.2 Marketing Strategies of Microgreens

The microgreen market is divided into segments based on the kind of predominant vegetable, the final product (beverages, food, cosmetics, etc.), and the means of distribution (supermarkets, restaurants, hypermarkets, etc.) (Paraschivu et al. 2021). Marketing microgreens effectively involves several strategic steps to ensure business growth and customer satisfaction. Firstly, developing a comprehensive business plan is crucial. This plan should outline the business goals, strategies, and financial projections, providing a roadmap for success. Establishing a strong online presence is essential. For instance in the US market most often utilized teaching resource for novice microgreens producers was the internet (Hamilton et al.

2023). Creating a professional website optimized for SEO can attract more traffic and potential customers (Fig. 4).

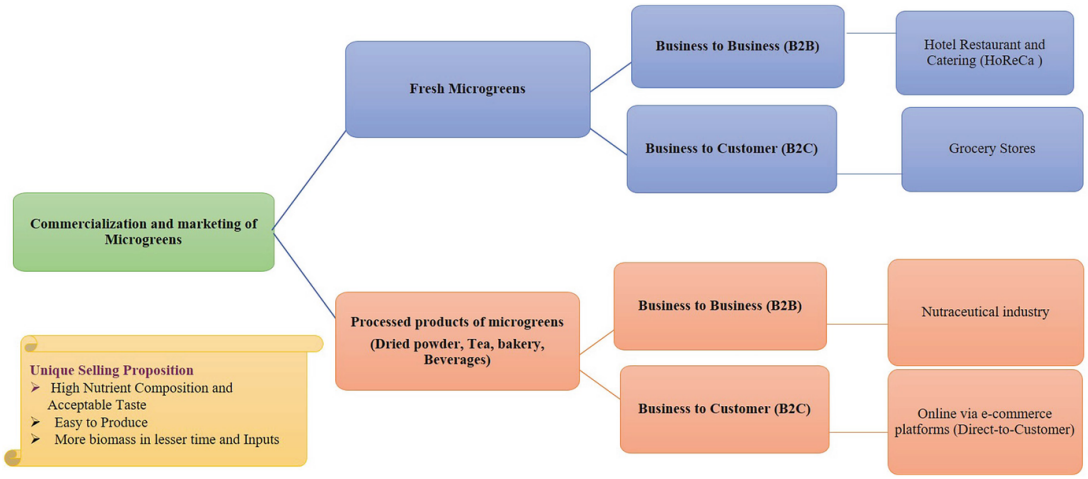


Fig. 4 Commercialization approaches for microgreens marketing

Additionally, setting up a Google My Business page and maintaining active social media profiles help in engaging with the target audience and building brand awareness. Identifying and understanding the target market is another critical aspect. For example in the US most microgreens were sold to restaurant operators for use in food service facilities (31.0%) or directly to customers at farmer's markets (32.7%) (Hamilton et al. 2023). Microgreens appeal to a diverse group, including health enthusiasts, gourmet chefs, and environmentally conscious consumers. Tailoring marketing messages to these segments can enhance customer engagement. Direct marketing to local restaurants, grocery stores, and farmers' markets is highly effective as these businesses often seek fresh and high-quality produce.

Muftiyatunnisa et al. (2023) has mentioned his findings, which showed that attention, interest, and search were all greatly impacted by social media. A major and beneficial influence on expanding one's understanding about microgreens goods is search. Purchase intentions for microgreens items are significantly influenced by one's level of knowledge about them.

Networking and collaboration are also valuable. Partnering with local chefs, nutritionists, and influencers can amplify the reach and credibility of the business. Attending food festivals, agricultural fairs, and local markets can increase visibility and provide opportunities for direct sales and feedback. By implementing these multifaceted marketing strategies, a microgreens business can thrive and achieve sustainable growth.

7 Applications of Bioinformatics in Healthcare, Pharmaceuticals and Nutraceutical Industries

Bioinformatics is a burgeoning multidisciplinary field that utilizes computational tools to analyse, visualize, organize, and store information related to biological macromolecules. Its primary focus is on obtaining biologically relevant data, such as DNA/RNA and protein sequences, structures, functions, pathways, and their interactions, which are then organized into databases. It has become indispensable in various domains, particularly in healthcare, pharmaceutical and nutraceutical industries.

7.1 Bioinformatics in Healthcare

The primary target of bioinformatics in healthcare is the analysis of large biological datasets to produce genomic and proteomic information essential for drug revelation and medication. Genomic medicine involves using genetic information to guide clinical care. Bioinformatics tools are used to analyse patients' data to diagnose genetic disorders, predict disease risk, and develop personalized treatment plans. This approach enhances diagnostic accuracy and treatment efficacy, paving the way for personalized medicine (Manolio et al. 2013; Sharma et al. 2021). Besides genetic diseases, NGS paired with bioinformatics has also been effectively utilized in a wide range of analyses relevant to public health infectious disease research. For instance, to identify outbreak sources, track transmission routes, study epidemic dynamics, determine disease-causing agents, and discover new human pathogens (Maljkovic Berry et al. 2020).

7.2 Bioinformatics in Pharmaceuticals

Prior to the advent of bioinformatics, researchers relied on analytical chemistry, pharmacology, and clinical sciences to identify new compounds, a method that was both time-consuming and expensive. Bioinformatics entered the pharmaceutical industry through two key areas: computational chemistry and molecular biology. Molecular biology offers a precise, targeted approach that focuses on genetics and data sequencing, essential for biomedical applications. In contrast, computational chemistry prioritizes structural analysis, including techniques like crystallography and protein structure determination, significantly aiding in the discovery and design of new drugs (Behl et al. 2021). By analyzing genomic and proteomic data, bioinformatics tools can identify potential drug targets. For instance, databases like DrugBank and PubChem provide valuable information on biomolecular interactions and drug activities, facilitating the identification of promising targets (Dibyajyoti et al. 2013). Computational methods such as molecular docking and molecular dynamics simulations predict how small molecules, such as potential drugs, interact with their target proteins. These methods help in optimizing drug candidates for better efficacy and reduced side effects (Salo-Ahen et al. 2021). Bioinformatics tools are also used to create predictive models that simulate biological processes. These models can predict how drugs will behave in the human body, including absorption, distribution, metabolism, and excretion (ADME) properties. By

using these simulations, researchers can anticipate potential issues and refine drug candidates before clinical trials, thereby reducing the risk of failure and the cost of development (Soliman et al. 2022).

7.3 Bioinformatics in Nutraceuticals

Today, increasing public awareness of health issues has led to a booming economy centred on food-derived bioactive compounds known as nutraceuticals. These compounds offer health-promoting and disease-preventing benefits. Nutraceuticals, which are gaining significant attention, represent a growing sector in both the pharmaceutical and food industries due to their beneficial functions. Nutrigenomics utilizes biochemistry, physiology, nutrition, genomics, proteomics, metabolomics, transcriptomics, and epigenomics to investigate and elucidate the intricate interactions between genes and nutrients at a molecular level. By analysing genomic data, bioinformatics helps understand how different nutrients influence gene expression and contribute to health and disease. This knowledge is pivotal for developing personalized nutrition plans and nutraceuticals tailored to individual genetic profiles, enhancing health outcomes and preventing diseases (Sales et al. 2014). Bioinformatics tools facilitate the discovery of bioactive compounds in foods that have health benefits. Databases and algorithms are used to mine biological data for functional ingredients, such as antioxidants, vitamins, and minerals, that can be developed into nutraceutical products. This accelerates the identification of new, effective compounds that promote health and well-being (Tsiaka et al. 2022).

7.4 Microgreens as Space Food

Microgreens were found beneficial in the culinary sector for many foods, due to their nutritional benefits and appetizing factors (Choe et al. 2018). Apart from possible health-promoting & culinary aspects, research into how microgreens are used in other fields is minimal. A notable benefit of microgreens is that, in higher altitude areas and extremely remote locations, where access to verdure could be severely hampered by transportation, growing microgreens could cater to a balanced diet for the local population (Singh et al. 2020). For instance, in the Trans-Himalayan region, a range of microgreens are carefully grown to provide the Indian armed forces with a highly nutritious food source, as providing nourishment at alpine can be challenging. They’ve produced a variety of microgreens, such as cauliflower, red cabbage, kohlrabi, radish, cabbage, and fenugreek, which have health-promoting phytochemicals and other necessary micro and macronutrients, while also considering the temperature and other environmental factors for the growth of microgreens (Singh et al. 2020).In a similar vein, it is feasible to grow nutrient-dense microgreens in space (Kyriacou et al. 2017). The concept of growing microgreens in space was inspired by extensive research and observation of astronauts to explore the

adaptability of human life in space. Microgreens require little time to cultivate and provide an abundance of nutrients. According to reports, astronauts suffer from several health problems as a result of their frequent travel and exposure to various environmental factors, including psychological stress, eye problems, damage to the central nervous system, weight loss from a lack of nutrient-dense diet, and more (Vergari et al. 2010; Cohu et al. 2014). To address these shortfalls, they considered several factors that influence microgreen growth and whether they could be optimized for better outcomes. According to the study's findings, astronauts may grow microgreens under specific conditions to suit their nutritional needs (Kyriacou et al. 2017). Microgreens, being a strong source of micronutrients, can also be utilized to improve cognitive function, which is associated with metabolic illnesses, particularly in the elderly population (Ozawa et al. 2021).

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8 Conclusion

Microgreens are functional foods that is small seedlings of various plants which are harvested 14–21 days after sowing and are rich in antioxidants, vitamins and minerals (iron, calcium, magnesium). But functional foods are those that not only offer vital nutrients, but also extra ingredients like phytochemicals or bioactive compounds that improve immunity, inflammation, digestion, and even lower the risk of some potentially fatal illnesses like cancer, diabetes, hypertension, and cardiovascular disease. As a result, microgreens are regarded as living, functional food that boosts the immune system's capacity to fend against illnesses and infections. Because of their antioxidant, anticancer, antibacterial, anti-inflammatory, anti-obesity, and antidiabetic qualities, they are a nutrient-dense addition to regular diets and supplements, and they are also being studied as medication in the pharmaceutical industry. During the growth of microgreens, it was observed in field and laboratory that there is infection and death of these tiny seedlings in early stages (data not published).Very limited literature is available related to microgreens cell death and immunity that can established molecular interactions. To identify the reason of cell death and its association with plant immunity will be very informative to explore the molecular processes and pathways for development of healthy and nutritious microgreens to fulfil the demand of safe food and its security. Leguminous plant microgreens are nutrient-dense powerhouses that include a wide range of macronutrients, micronutrients, and phytochemicals. Eating them is a quick and effective way to increase the amount of important nutrients that are ingested. Leguminous microgreens are a concentrated source of essential nutrients that can improve general health and well-being when added to diet. To learn more about the bioavailability and possible health advantages of these nutrient-rich microgreens, more investigation is necessary.

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Phytochemical and Nutritional Properties of Leguminous Microgreens and Its Application in Food Industry

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Abbreviations

DPPH 2-Diphenyl-1-picryl-hydrazy
GC/MS Gas Chromatography-Mass Spectrometry
QMP Pectic Polysaccharides from Quinoa Microgreens
TAA Total Antioxidant Activity
TAC Total Ascorbic Acid
TFC Total Flavonoids Content
TPC Total Phenolics Content

1 Introduction

Microgreens are the colorful, nutrient-dense seedlings of a wide variety of vegetables, oil-seeds, culinary and medicinal herbs that have become incredibly popular in recent years because of their remarkable nutritional profile and flavor (Appolloni et al. 2022). These little greens are incredibly rich in macro as well as micro nutrients and packed full of vital active biological compounds like carotenoids, phenols, glucosinolates etc. despite their diminutive size. Additionally, they are known for their bland to tart flavor.

Microgreens are herb seedlings that are soft, crisp, fresh, and fragrant. Chefs and home cooks alike admire their delicate yet colorful appearance, which adds different textures, flavors, visual appeal to a variety of culinary preparations. This variety not only improves the whole eating experience, but it also encourages people to include more plant-based foods in their diets, which

promotes overall health and sustainability. Furthermore, they have a higher nutritional value than mature leafy greens (Bulgari Flores et al. 2024).

In addition to their culinary uses, microgreens have sparked attention in the field of sustainable agriculture. Their capacity to be grown indoors year-round, with limited space and resources, makes them an appealing option for urban farming and community gardening efforts (Bulgari et al. 2022). Microgreens help to create more resilient and sustainable food systems by increasing local food production and lowering the environmental imprint of traditional agriculture (Zhang et al. 2021).

2 Microgreens—What Makes Them Potential Functional Food?

Research has shown that microgreens can have 4–40 times more nutrients than fully-grown vegetables (Weber 2017). Furthermore, the expanding quantity of scientific evidence supporting the health advantages of microgreens has piqued consumers' attention, resulting in increased demand for these nutrient-dense greens. The health implications of metabolites found in microgreens of various plant species has been studied. For instance, pectic polysaccharides are a group of health-efficacious metabolites present in microgreens of quinoa. These pectic polysaccharides sourced from quinoa microgreens (QMP) possess anti-glycation response, immunostimulatory effect, antioxidant potential as well as prebiotic capacity (Li et al. 2024). QMPs in their native and de-esterified forms (varied degrees of de-esterification) activate TLR4/NF-κB signaling pathway, thereby resulting in immunostimulatory response. Similarly, microgreens of Brassicaceae members —cauliflower, red mustard, broccoli have significant amounts of health beneficiary glucosinolates and phenol containing compounds (García-Pérez et al. 2024).

Microgreens are also being explored as potential supplements during space exploration missions based on their sensory attributes, storability and prospective impact on physical and health of astronauts (Teng et al. 2023). As knowledge of the importance of nutrition and preventive health measures continues to develop, functional foods like microgreens will likely become increasingly attractive alternatives for health-cognizant persons trying to optimize their diets. Therefore, microgreens are considered potential functional foods that can promote human health and well-being (Di Gioia et al. 2021). The difference in concentration of nutrients/phytochemicals from seed to fully grown plants is depicted in Table 1.

Table 1 Comparison of nutrient or phytochemical content in different stages of a plants' life cycle-seed, baby greens, microgreens and mature plant

Crop	Nutrient/phytochemical	Microgreens	Mature plants	References
Cucumber (*Cucumis sativus* L.)	Vitamin C/ascorbic acid	34.90 mg/100 g of fresh weight	10.00 mg/100 g of fresh weight	Zhang et al. (2021); Rani and Singh (2021); Laila et al. (2022); Sofi et al. (2023); Reddy et al. (2021)
Jute (*Corchoris olitorisu* L.)		25 mg/100 g of fresh weight	17.45 mg/100 g of fresh weight	
Sicilian broccoli (*Brassica oleracea var. italica*)		7.5 mg/g	–	
Buckwheat (*Fagopurum tarticum*)		3.0 ± 0.0 µg/100 g of fresh weight	2.0 ± 0.0 µg/100 g of fresh weight	
Fenugreek (Trigonella *foenum-graecum*)		5.00 ± 0.0 µg/100 g of fresh weight	3.0 ± 0.0 µg/100 g of fresh weight	
Purple cabbage (*Brassica oleracea var. capitata f. rubra*)		147.0 mg/100 g FW	24.4 mg/100 g FW	
Choy sum (*Brassica rapa subspecies chinensis var. parachinensis*)	Vitamin K/phylloquinone	377 ± 29 µg/100 g of fresh weight	363 ± 27 µg/100 g of fresh weight	Zou et al. (2021)
Borage (*Borago officinalis L.*)	Vitamin E/tocopherol			
Borage—Bor 1	α-Tocopherol	5.45 µg/g of fresh weight	2.49 µg/g of fresh weight	Mallor et al. (2023)
	γ-Tocopherol	1.55 µg/g of fresh weight	0.10 µg/g of fresh weight	
	δ-Tocopherol	3.32 µg/g of fresh weight	0.04 µg/g of fresh weight	
Borage—Bor 2	α-Tocopherol	4.90 µg/g of fresh weight	2.77 µg/g of fresh weight	
	γ-Tocopherol	1.61 µg/g of fresh weight	0.14 µg/g of fresh weight	
	δ-Tocopherol	2.91 µg/g of fresh weight	0.05 µg/g of fresh weight	
Red Cabbage (*Brassica oleracea L. var. capitata f, rubra*)	Lutein/zeaxanthin	28.6 mg/100 g of fresh weight	8.6 mg/100 g of fresh weight	Ebert (2022)
Coriander (*Coriandrum sativum*)		11.2 mg/100 g of fresh weight	10.1 mg/100 g of fresh weight	

Crop	Nutrient/phytochemical	Microgreens	Mature plants	References
Choy sum (*Brassica rapa subspecies chinensis var. parachinensis*)	Aliphatic glucosinolate	517 ± 125 µg/100 g of fresh weight	85 ± 16 µg/100 g of fresh weight	Zou et al. (2021)
Chicory (*Cichorium intybus L.*)	β-Carotene			
Chicory—Chi 1		59.48 µg/g of fresh weight	51.62 µg/g of fresh weight	Mallor et al. (2023); Rani and Singh (2021)
Chicory—Chi 2		55.04 µg/g of fresh weight	49.55 µg/g of fresh weight	
Broccoli (*Brassica oleracea var. italica*)		1763.33 ± 3.51 µg/100 g of fresh weight	58.67 ± 0.58 µg/100 g of fresh weight	
Lettuce (*Lactuca sativa*)	Carotenoids			
Lettuce—Let 1		140.54 µg/g of fresh weight	126.99 µg/g of fresh weight	Mallor et al. (2023); Zhang et al. (2021)
Lettuce—Let 2		161.95 µg/g of fresh weight	116.03 µg/g of fresh weight	
Wheat (*Triticum aestivum*)		53.36 mg/100 g DW	–	
Barley (*Hordeum vulgare*)		56.08 mg/100 g DW		
Red Salanova butterhead lettuce (*Lactuca sativa L. var. capitata*)	Polyphenols	1086 ± 25.8	526 ± 22.8	El-Nakhel et al. (2020)
Green Salanova butterhead lettuce (*Lactuca sativa L. var. capitata*)		314 ± 0.68	230 ± 5.73	

3 Different Groups of Microgreens

Importance of microgreens as a nutritious addition to daily diets, supplements, and other culinary activities has already been discussed in previous sections. The major advantage of microgreens production is that they are not limited to any specific crop and can be widely grown from different crop families adding diversity in nutritional value, color, flavor, aroma and taste. This provides a combination of crop specific phyto-chemicals on the platter for diet

enrichment. The mature leaves of only a few plants can be eaten as they have little acceptability for direct or raw consumption due to their astringent or bitter taste contributed by high content of phenolic compounds, tannins etc. However, this limitation can be overcome at the stage of microgreens. Therefore, increasing number of studies have focussed on the comparative analysis of phyto-chemical composition and sensory acceptability of microgreens from diverse group of plants (Gunjal et al. 2024; Bulgari Flores et al. 2024; García-Tenesaca et al. 2024).

3.1 Microgreens from Vegetables

Microgreens in general are referred to as edible vegetables, however they are produced from various vegetable crops like beetroot, broccoli, radish, mustard, swiss chard, rocket (arugula), kale, celery etc. (Fig. 1). Vegetable microgreens bring together a diverse group of plants' chemical composition thus enriching the diet. For example, microgreens of radish have been reported to accumulate high levels of alpha-tocopherols, iso-thiocyanates, vitamin C, omega-3 fatty acids, carotenoids along with minerals like magnesium, selenium, calcium and phosphorus (Sharma et al. 2022). Similarly, microgreens of family Brassicaceae are rich in glucosinolates (GLs) and flavonoids—compounds with profound antioxidant capacity, imparting them potential to combat chronic health conditions like inflammation, cardio-vascular ailments etc. (Zhong et al. 2024).



Fig. 1 Microgreens of different crop varieties viz. beetroot, brassica, chickpea and maize (left to right). Each of these microgreens have distinct colour (hence visual appeal), flavor, taste and mouth feel. Further, each of these posses characteristic nutrient content

3.2 Microgreens from Cereals and Pseudocereals

Edible grains like rice, millets, wheat, maize belonging to family poaceae are grouped as cereals while those like chia, quinoa, amaranth etc. belonging to other families are referred to as pseudocereals. Cereals especially whole grain cereals have starch rich endosperm and are prominent source of vitamin B (niacin, riboflavin), vitamin E, lutein, carotenoids, polyphenols-flavonoids, phytosterols, anthocyanidins and dietary protein (Gunathunga et al. 2024). Likewise, pseudocereals are profound sources of proteins with an optimized concentration of amino acids, crude fiber, minerals (iron and calcium) and compounds with antioxidant efficiency like phenolics etc. (Anberbir et al. 2024). Both cereals and pseudocereals are reservoirs of nutrition and phyto-active compounds, adding to their abundant anti-oxidant capacity. To combine the nutritional aspects, microgreens of these two groups of plants are increasingly studied. For instance, numerous investigations have been

focussed on microgreens of buckwheat, barnyard millet, red amaranthus, quinoa and chia (Rani et al. 2024; Durairajan et al. 2024; Gunjal et al. 2024; Li et al. 2024; Wu et al. 2024; Gök et al. 2024).

3.3 Microgreens from Oil Seed Crops

Oleaginous crops like canola/rapeseed, sunflower, soy, chia and flax seed have received special attention for microgreens' production because of their distinctive bioactive composition that includes their fatty acid profile. Studies have also focussed on their sensory attributes and post-harvest management (Dalal et al. 2020; Dalal and Siddiqui 2019). Castellaneta et al. 2024 utilized mass spectrometry coupled gas chromatography to discover the wholesome fatty acid profile of oleaginous microgreens of soy, canola, chia, sunflower and flax.

3.4 Microgreens from Legumes

Legumes including lentils, chickpea, soybean, cowpea are members of family fabaceae, characterized by invariably high protein content thus making them richest source of botanicals' derived protein (Gunathunga et al. 2024). While they are abundant in vitamins, minerals, dietary fibers and polyunsaturated fatty acids they lack cholesterol and saturated fatty acids (Röös et al. 2020). Microgreens of pea and mung bean grown in elevated altitudes of India were found rich in beta-carotene, vitamins (A, B_3, B_5, and C) and minerals like iron and manganese (Rani et al. 2024).

3.5 Microgreens from Medicinal Herbs

Traditionally, herbs have found diverse therapeutics and culinary applications owing their anti-oxidant, anti-microbial, anti-inflammatory, anti-parasitic properties, flavor and aroma (Fig. 2). Herbs like *Ocimum sp.*, *Tribulus terrestris* have been regarded as 'functional foods' since time immemorial as they possess active plant chemicals in abundant quantities (Kumari et al. 2024). Furthermore, metabolic profiling of fenugreek microgreens (a leguminous herb) revealed peak in concentrations of flavonoids, tannins and phenols (Jayaraman and Ramasamy 2024). Similarly, metabolite profile of basil microgreens has been studied under different conditions of light period (Kumar et al. 2024).

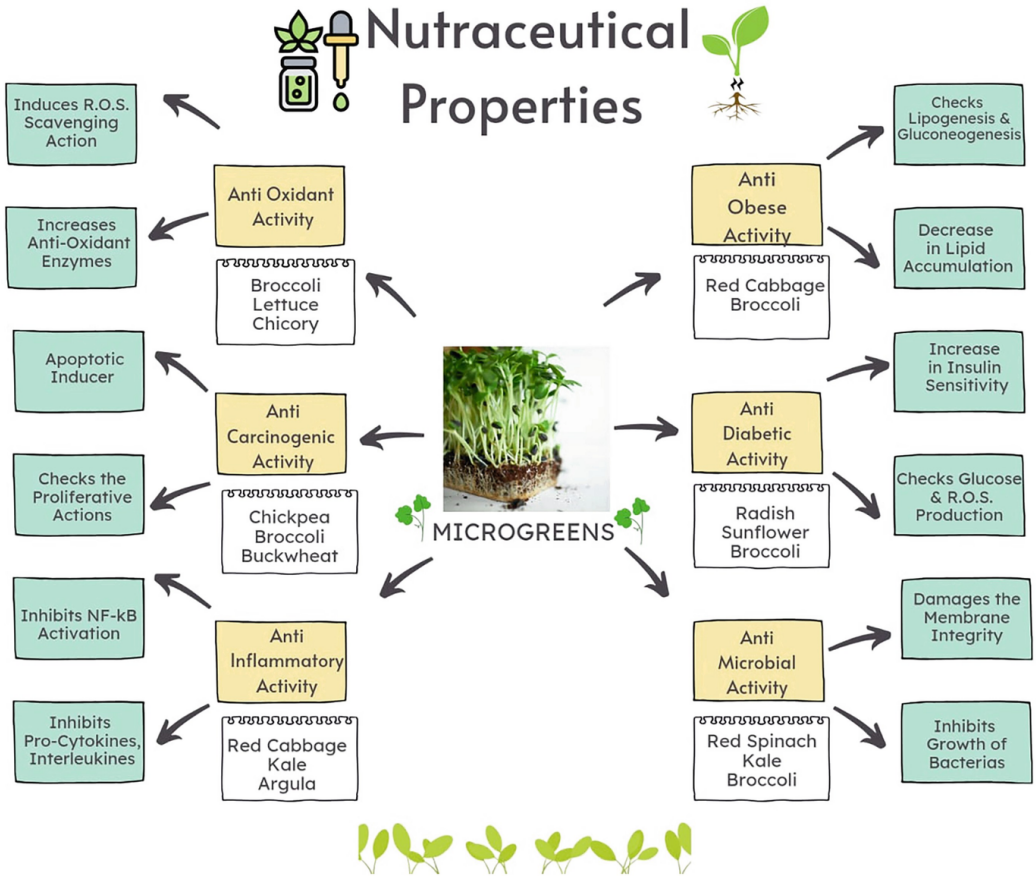


Fig. 2 This diagram illustrates the diverse nutraceutical properties of various microgreens, highlighting their specific biological activities and associated health benefits. The central image represents microgreens, emphasizing their pivotal role in providing these health benefits through various biochemical pathways and mechanisms. The diagram underscores the importance of incorporating microgreens into the diet for their multifaceted therapeutic effects

4 Germplasm or Varieties with Desired Nutritive Traits

Legumes or pulses are members of Fabaceae and are second most important grain crops after cereals. The diversity in profile of nutrients like proteins, dietary fibre, carbohydrates and minerals essentialize their role in nutritional security, simultaneously enriching soils' nutrient profile and ecological sustainability via nitrogen fixation (Gayacharan et al. 2023). They are also regarded as 'poor man's meat' as the vegetarian population is largely dependent on them for fulfilment of their protein requirement thus making them a sustainable source of protein (Semba et al. 2021). Several crop improvement strategies have been devised to enhance the nutritional quality and composition of grain legumes thereby serving as a source to combat hidden hunger. Considering their diverse nutritional qualities leguminous crops can be a potential candidate for microgreens cultivation. Further, the diversity in available germplasm can be explored to produce microgreens possessing desirable or enhanced nutrient traits.

4.1 Available Germplasm or Varieties with Desired Nutritive Traits

The genetic variety seen in plant species, including differences in characteristics like yield, disease resistance, and nutritional makeup, is referred to as germplasm. Germplasm, the genetic material of plants, plays a crucial role in agricultural development, particularly in breeding programs aimed at enhancing the nutritional quality of crops (Khan et al. 2022). With advancements in genetic research and breeding techniques, scientists have been able to identify and develop plant varieties with desired nutritive traits to address nutritional deficiencies and promote better health outcomes.

Microgreens as a food supplement are known to regulate weight growth, cholesterol metabolism, and heart disease prevention. Leguminous microgreens are regarded as a unique product for dietary diversification since they are high in vitamins, antioxidants, and minerals, among other phytochemicals (Priti et al. 2021). Because of their rich nutritional profile and possible health advantages, legume microgreens provide a promising new direction in the field of functional foods. These microgreens provide a rich variety of germplasm that may be utilized for breeding initiatives and genetic improvement. They are derived from leguminous plants including mung beans, chickpeas, and lentils (Parida et al. 2023). By delving into leguminous germplasm resources, we can find important genetic variants that support increased stress tolerance, nutritional content, and productivity simultaneously.

Breeders can create cultivars that meet specific dietary needs and consumer preferences by discovering and describing distinctive genetic features present in leguminous microgreens. These cultivars will also have optimum agronomic qualities and nutritional profiles (Parida et al. 2023). Furthermore, the capacity to grow microgreens in a variety of geographical locations is made possible by the diversity of leguminous germplasm, which supports efforts to promote sustainability and global food security. There are different types of microgreens available at commercial scale, and if we specifically talk about leguminous microgreens than some very common microgreens like chickpea, lentils, mungbean, pea etc. are very known and widely available.

4.2 Pea Shoots (*Pisum sativum*)

Pea shoots are highly valued for their delightfully crisp texture and somewhat sweet flavor, and they are harvested from the seeds of *Pisum sativum*. These early-growing microgreens add a nutritious boost and a blast of freshness to a variety of recipes (Ebert 2022). Pea shoots are high in vitamins, minerals, and antioxidants that promote general health and a balanced diet. They are a flexible culinary addition that go well with salads, sandwiches, stir fries, and garnishes because of their subtle flavor. Pea shoots are also a popular option for both commercial farmers and household gardeners since they are simple to grow both indoors and outdoors. Their bright flavor and texture enhance culinary experiences, and their quick growth and large output make them a reliable and easily available supply of fresh greens all year long.

4.3 Diversity of Small Seeded and Large Seeded Legumes

There exists a vivid diversity in seed size amongst leguminous crops, even within the same group. For instance, seed size particularly seed diameter plays a major determinant in overall quality of lentil and consumer acceptance (Singh et al. 2022). Seed diameter of large seeded lentils or macro-sperma i.e. Chilean type seeds range up to 0.5 inch (6–9 mm), on the other hand diameter of small seeded lentils or micro-sperma i.e. Persian type range up to 0.25 inch (2–6 mm) (Dutta et al. 2023). Further, the morphological features of cotyledons also show variation to the extent that macro-sperma have yellow coloured cotyledons while cotyledons of micro-sperma exhibit yellow, orange and red colours (Dutta et al. 2023). Lentil genotypes L830 and L4602 demonstrate significant difference in seed diameters therefore are regarded as small seeded and large seeded, respectively. Additionally, documented 1000 seed weight of L830 is 20.9 grams and that of L4602 is 42.13 grams.

The variation in seed size of plants, particularly legumes has occurred during the course of crop domestication as reported in several beans, lentils etc. (Dutta et al. 2023; Cuny et al. 2017). As a consequence of domestication, profound evolution of seed size, shape and content of certain toxic compounds like cyanogenic compounds, lectins as well as lectin like proteins as in case of *Phaseolus lunatus,* has occurred.

Similarly, chickpeas (*Cicer arietinum*) have been categorised as macro-sperma—the kabuli type and micro-sperma—the desi type, based on size of their seed and morphology (Sankaran et al. 2016). Kabuli type seeds are shaped like an owl head, larger in size and lighter as compared to desi type seed which are shaped like a tear drop. Additionally, seeds of chickpea have been classified by the USDA National Agricultural Statistics Service (NASS, 2015) on the basis of their diameter as small-diameter <7.9 mm and large-diameter ≥7.9 mm.

The features of small-seeded legumes are remarkably diverse, providing a multitude of alternatives for both agricultural and gastronomic use (Semba et al. 2021). This variety includes variances in development patterns and environmental tolerance in addition to differences in seed size, shape, and nutritional makeup.

Legume plants with small seeds provide a variety of ways to produce microgreens, which adds to the increasing demand for these tasty and nutrient-dense greens. Recent trends demonstrate the potential of small-seeded legumes for microgreen growing, such as *Onobrychis viciifolia*, *Medicago sativa*, and *Trifolium pratense*, and offer insightful information about their nutritional makeup and health advantages (Gupta et al. 2023). These plants' adaptability makes it possible to create colorful and diverse microgreen mixtures that enhance nutritional and gastronomic experiences.

This variety in seed size and shape as demonstrated by legumes like *Medicago sativa* (alfalfa), *Trifolium pratense* (red clover), and several kinds of lentils and beans, each of which has special qualities that add to their importance as agronomic crops and food sources (Chiriac et al. 2020). Nutritionally speaking, small-seeded legumes are vital parts of healthy diets all over the world since they are excellent providers of protein, dietary fibre,

vitamins, and minerals. As Partap et al. (2023) have noted, factors like seed sowing density are critical to maximizing microgreen development and resource use. Furthermore, microgreens made from small-seeded legumes serve as plant-based, nutrient-dense vegetarian and vegan meals that have the potential to improve dietary diversity and health while also promoting nutritional security (Dhaka et al. 2023).

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5 Significance of Microgreens in Food Industry

In recent times, microgreens have gained popularity as novel foods and edible micro-scale vegetables with extraordinary texture, appearance, flavor, colour and density of nutrients. These super greens contribute to diversity in diets of vegetarians, vegans and space hawkers (Kathi et al. 2024). Being recognised as foundation of plant-based diet, they can be consumed fresh and raw after harvest or used to garnish dishes, in soups, salads, shakes etc. (Maru et al. 2024). Owing to their significant characteristics, they have been included in 'gourmet cuisine', where these have been referred to as 'confetti' and 'lingerie of culinary world' (Bulgari Flores et al. 2024; Verlinden 2020).

In addition to their consumption as discussed above, microgreens and their extracts can also be incorporated in a variety of food product preparations. This brings together the aroma, flavor, colour along with the nutrient package of microgreens into a more acceptable and readily consumable value-added food product. In this light, a compendium of microgreens or microgreens powder from variety of plant sources alone or in combination may be used in food processing sector such as confectionery, bakery etc. highlighting their potential utility as an additive to food and food products (Sanyukta et al. 2023). Further, microgreens may even serve as source of "fortification" in food products.

5.1 Ready-to-Serve Beverages

In fast paced life, there has been a growing demand and consumption of ready to consume food products such as ready-to-eat foods and ready-to-serve beverages. Ready-to-serve drinks are pre-packed beverages composed of fruit extracts, sugar or sweeteners, along with water and acid (Chandola 2024). Thus, available for direct consumption these drinks serve as source of refreshment while offering diverse flavors in a time saving and convenient manner. In purview of this, research has seen growing surge in development of value added ready-to-serve functional drinks derived from extracts of fruits, vegetables as well as medicinal herbs with acceptable palatability (Pillai et al. 2024). This combines together the therapeutic, nutritional and medicinal aspects of derivative botanicals.

Potential of microgreens can be harnessed in making beverages like juices—both vegetable and fruit based, fermented drinks etc. labelling them as vital resource for development of nutritionally rich and fortified food products. Addition of extracts from microgreen of specific plant species, example brassica, kale and many other, includes essential health-benefitting phyto-active compounds particular to those plant species (Sehrish et al. 2023). Thus,

concentrations of secondary metabolites and nutrients are enhanced in the food products. Some notable food products produced using microgreen powders include probiotics, yogurt, flour, breads, tea etc. (Singh et al. 2024).

5.1.1 Microgreen as Tea

Herbal tea—the infusions of botanicals such as plant roots, leaves, flowers etc., have attracted popularity for maintaining healthy lifestyle as profound source of nutrients and phyto-chemicals including antioxidant compounds while being devoid of caffeine (Poswal et al. 2019). Further, consumption of herbal tea may be regarded as immunity promoter as it helps build shield against several life-threatening diseases like that of heart, nervous system, cancer etc. Herbal tea has previously been prepared from *Melissa officinalis* L., commonly known as lemon balm owing to its promising health attributes. Consequently, microgreens have emerged as reservoirs of flavours, nutrients and minerals. Thus, lemon balm microgreens were used for preparation of herbal tea to analyse their phenolic, antioxidant and mineral profile (Newman et al. 2023). Further, these nutrients' profile was compared with herbal tea prepared from its' adult stage and the effect of cold and hot brewing on nutritional quality was assessed.

5.1.2 Bakery Products: Muffins and Cake

Bakery products are favourite delicacies of people of all age groups. However, the calories associated with their sugar content and other ingredients have limited their consumption among people with diabetes, other health-related conditions and health-conscious population. Microgreens powder supplemented bakery products would enable their acceptance and consumption among these groups also. Functional muffins have been prepared with microgreens juice powder (MJP) of basil, fenugreek and wheat grass (Kumar and Singh 2024). Similarly, gluten-free cakes have been prepared by utilizing sunflower microgreen powder as flour (Mansouri et al. 2024).

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6 Conclusion

Microgreens are nutritious juvenile greens harbouring a wide range of essential bio-active compounds with remarkable health beneficiary properties. Health related benefits of these young greens include immunity enhancing, anti-cancerous, anti-diabetic and anti-inflammatory properties among many others. The liberty to produce microgreens from different plant groups like cereals, pseudo-cereals, legumes, medicinal herbs, oil seed crops etc. helps to amalgamate the range of nutrition profile of these plant groups together providing a wholesome bowl of nutrition for consumers. Simultaneously microgreens have also found application in food industry where they may be incorporated into dishes fresh as well as processed. The incorporation of microgreens in diet is equally essential for human well-being. Further, formulation of food products utilizing microgreens—fresh or processed (dry) in food processing industry is an arena of great interest.

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