

BIOFORTIFICATION IN PULSES AND LEGUMES FOR ALLEVIATING MALNUTRITION AND ENHANCING NUTRITIONAL SECURITY: A REVIEW

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Abstract. The global population is expected to reach 8.6 billion by 2030 and 9.8 billion by 2050. Micronutrient malnutrition, resulting from an unbalanced diet, is a major issue leading to severe socio-economic consequences such as stunting, wasting, marasmus, kwashiorkor, anemia, environmental enteric dysfunction, and impaired immunity, particularly in low-income and developing regions like South Asia and sub-Saharan Africa. Promoting global food stability and improving the nutritional quality of food crops are potential strategies for enhancing the human immune system. Pulses and legumes, rich in essential vitamins, minerals, and complex carbohydrates, are key contributors to human growth and development. Biofortification, achieved through agronomic techniques, traditional breeding, or biotechnological advancements, involves increasing nutrient levels or accessibility in staple food crops. This process not only improves crop yields and nutritional value but also alleviates micronutrient deficiencies in consumers. This review comprehensively explores the nutritional importance for human health, the current status of malnutrition, and various biofortification approaches for enhancing nutritional quality in pulses and legumes.

Keywords: *malnutrition, micronutrients, biofortification approaches, pulses and legumes*

Introduction

Several developing nations are contending with silent epidemics of nutritional deficiencies in both humans and animals, exacerbated by lack of dietary diversity, particularly in cereal-based crops with insufficient mineral nutrients (Dhaliwal et al., 2022). It is estimated that by 2030, 840 million individuals will be undernourished globally (FAO, 2020a). Additionally, over 2 billion individuals worldwide face deficiencies in key micronutrients such as iron (Fe) and zinc (Zn) (Huang et al., 2020). Worldwide, Zn and Fe deficiencies have affected one-fifth and one-third of the population, respectively (Kumar et al., 2017). Micronutrient deficiencies contribute to malnutrition leading to severe socio-economic consequences like stunting, wasting,

marasmus, kwashiorkor, anemia, environmental enteric dysfunction and impaired immunity, especially prevalent in low-income and developing regions like south Asia and sub-Saharan Africa (Sheoran et al., 2022; Borresen et al., 2017). Many individuals, particularly infants fail to fulfill their daily protein requirements, impacting overall growth and development (Müller and Krawinkel, 2005; Borresen et al., 2017).

Nutritional security, denoting the intake of food enriched with essential nutrients, is a critical consideration, especially in developing countries (Maertens et al., 2017). Hidden hunger persists, even with carbohydrate-rich diets, due to unmet micronutrient requirements (Bouis et al., 2018). Addressing malnutrition requires dietary patterns that incorporate vital nutrients such as carbohydrates, fats, proteins, vitamins, and minerals (Lean, 2019). Given its severe consequences, eradicating malnutrition is crucial for a healthy world (Jangir et al., 2017; Kumar et al., 2017). The global community, through the Sustainable Development Goals (SDGs) established in 2015, that aims to eliminate malnutrition in all its forms, particularly through SDG2, “Zero Hunger,” and SDG3, “Good Health and Well-Being” (Global Nutrition Report, 2017; Hawkes, 2017). Promoting global food security and fortifying food crops represent potential strategies for boosting human immunity. Grain legumes, commonly known as the “poor man’s meat,” emerge as a plentiful source of plant-based protein in high demand. They provide crucial amino acids and address the increasing requirement for protein-rich diets (Hall et al., 2017; Singh et al., 2017; Hou et al., 2019). Pulses and legumes, being rich in vitamins, minerals, and complex carbohydrates, play a crucial role in optimal growth and development (Tuso et al., 2013). Given the severity of its impacts, the only lasting solution for fostering a healthy world is the eradication of malnutrition (Jangir et al., 2017; Kumar et al., 2017). Biofortification is a sustainable and economically viable method to enhance both crop yield and quality, for alleviating malnutrition and hidden hunger among the world (Dhaliwal et al., 2022).

Methodology

This review was compiled, processed and manipulated at the Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore from October 2023 to March 2024. This manuscript holds out the discussion of reviews from more than two hundred scientific research papers. Sources like, TNAU e-library, Scopus, ARCC journals, Google Scholar, Research Gate, were utilized for review writing (keywords: malnutrition, micronutrients, biofortification approaches, pulses and legumes).

Assessing micronutrient malnutrition worldwide: a global perspective

Globally, one-fifth and one-third of the population suffer from Zn and Fe deficiencies, respectively, with zinc deficiency being widely spread in developing nations (Kumar et al., 2017). Hidden hunger, the inconspicuous manifestation of micronutrient malnutrition, affects one in three individuals globally, with significant concerns surrounding deficiencies in Vitamin A, Iron (Fe), Iodine (I), Zinc (Zn), and folate. The conceptualization of malnutrition traces back to the early 20th century, with the 19th century already recognizing the health significance of trace elements such as Fe, Zn, and I (Dhaliwal et al., 2022). Micronutrient deficiencies globally impact around 38% of pregnant women and 43% of preschool-aged children, contributing to anemia in exceeding 30% of the global population (Stevens et al., 2013). Nearly half of the

world's population faces the dual challenge of micronutrient deficiency and undernourishment during pregnancy, resulting in possible negative consequences like intrauterine growth restriction, low birth weight, protein-energy malnutrition, and chronic energy deficit (Ahmed et al., 2013). Malnutrition, as per the definition provided by the World Health Organization (WHO), encompasses discrepancies in individuals' dietary intake, leading to deficiencies or excesses. This encompasses two main concerns: A) undernutrition cause stunting, wasting, underweight, and B) overnutrition cause overweight and obesity (*Table 1*).

Table 1. Different forms of under and over nutrition

S. No	Nutrient deficiencies	Meaning	Symptoms	References
Undernutrition				
1.	Stunting	Low "height for age" is known as stunting	Reduced height	Siddiqi et al., 2023
2.	Wasting	Low "weight for height" is referred to as wasting	Lower weight	
3.	Kwashiorkor (edematous malnutrition)	It is a form of malnutrition caused by a lack of protein in the diet	Fatigue, diarrhea, loss of muscle mass and irritability	Benjamin and Lappin, 2018
4.	Marasmus	Protein energy malnutrition, mainly seen in children	Weight loss, stunted growth, diarrhea, lower immunity, stomach infection and lactose intolerance, respiratory infections, dry skins and eyes, brittle hair	www.nipccd.nic.in
Overnutrition				
5.	Overweight/obesity	Obesity is a chronic complex disease defined by excessive fat deposits that can impair health	Increased risk of type 2 diabetes and heart disease, it can affect bone health and reproduction, it increases the risk of certain cancers	Okunogbe et al., 2022

The Global Hunger Index 2023 ranks India 107th out of 121 countries, with a score of 29.1, indicating a serious level of hunger (20.0-34.9) (Thakur et al., 2023). Globally, 633 million people, constituting 8.9% of the world's population, do not meet their daily calorie intake, leading to undernourishment. Among children aged under 5 years, 22 out of every 100 are stunted, reflecting insufficient growth compared to their peers. Severe food insecurity affects 9% of the global population, with 1 in 4 individuals experiencing moderate food insecurity, totaling 1.9 billion people. The UN Sustainable Development Goals aim to "End hunger by 2030," but insufficient progress has been made towards this target. Despite South Asia's status as one of the fastest-developing regions, it encounters a paradoxical scenario regarding malnutrition. Approximately 33.3% and 15.3% of children under the age of five experience moderate to severe stunting and wasting, respectively, while 3.1% of children suffer from overweight conditions (Akhtar, 2016a). Various malnourishment in children under 5 by regions (%) given in *Figure 1*.

The effects of malnutrition on human health: unveiling nutritional challenges

Micronutrient deficiency is linked to various physiological impacts, including impaired physical and intellectual growth in children, anemia, and maternal mortality resulting in compromised cognitive functions, along with disorders such as blindness and decreased productivity (Akhtar, 2016b). An alarming 88% of countries across Asia and Africa are grappling with two or three types of malnutrition concurrently, attributed to insufficient food availability, limited household incomes, inadequate healthcare

infrastructure, inadequate childcare practices, and food insecurity. Various effects of micronutrient malnutrition on human health is given in *Figure 2*. Micronutrient-deficient in agricultural products contribute to poor health, enhance the morbidity and disability, stunted mental and physical growth, perinatal complications, impaired development, diminished livelihoods, and reduced national socio-economic development and quality of life (Bailey et al., 2015). Additionally, they exacerbate infectious and chronic diseases, including osteomalacia, osteoporosis, thyroid deficiency, colorectal cancer, and, cardiovascular diseases, significantly impacting the quality of life (Tulchinsky, 2010). Uneven distribution of nutrients among different plant parts is another significant consideration (Zhu et al., 2007). Deficiencies of Iron (Fe), Zinc (Zn), folic acid, and Beta-carotene are global issues, particularly predominant in Asia, Africa, and Latin American countries, affecting more than 2 billion peoples (Tulchinsky, 2010; Darnton-Hill et al., 2006).

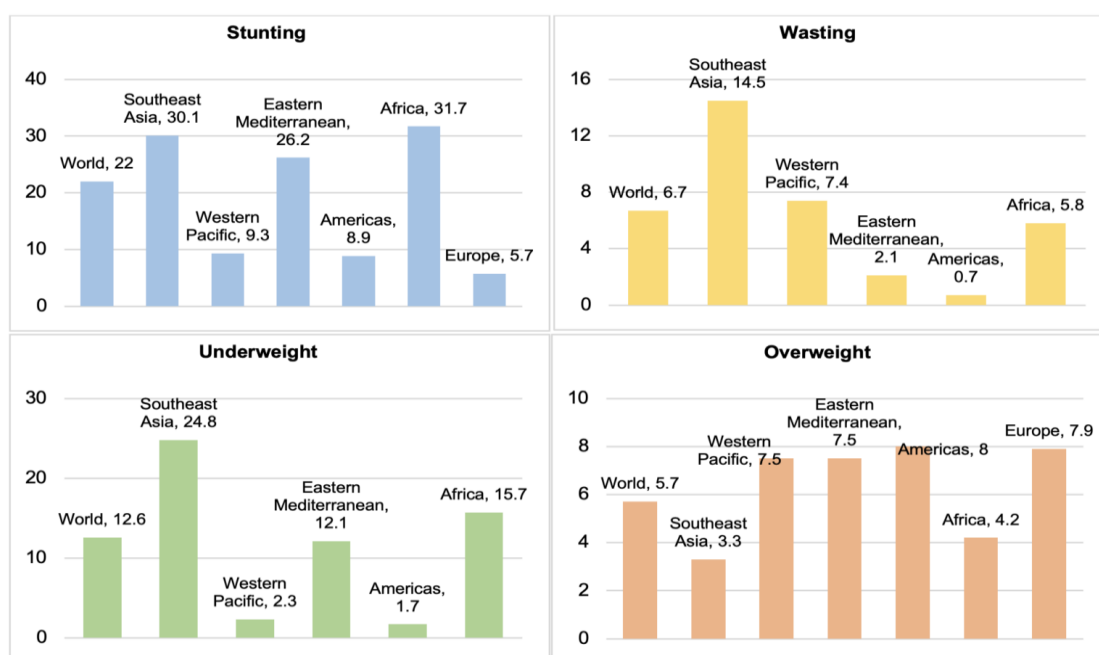


Figure 1. Prevalence of malnourishment in children under 5 by regions (%) WHO, GHO, Nov 2022. Data source: WHO - World Health Organization. GHO - Global Health Observatory

Bio-fortification

The global spotlight on micronutrient malnutrition has prompted initiatives to address it through diverse strategies, including increased food production, supplementation, food fortification, and bio-fortification. The concept of biofortification originated in the period of green revolution (1966–1985), with the term “biofortification” coined by Steve Beebe in 2001. Recognizing the focus on increasing crop production and productivity while neglecting the nutritional status of crop cultivars and human health, biofortification has gained prominence as a means to address hidden hunger or micronutrient malnutrition, especially in developing countries (Khush et al., 2012). The Copenhagen census highlights the significance of reducing malnutrition, ranking biofortification as the fifth main area to invest in to address this problem (Kumar and Pandey, 2020). The shift in agriculture towards

producing nutrient-rich food crops, in addition to increasing quantity-wise production, is seen as a crucial step in fighting hidden hunger, Especially, prevalent in impoverished and developing nations, where diets are primarily composed of staple food crops lacking in micronutrients (Khush et al., 2012). The harvest plus program is actively contributing to biofortification by enhancing both nutrient and yield traits (Unnevehr et al., 2007). Biofortification nutritional goals include enhancing the mineral and vitamin content, elevating essential amino acid levels, improving fatty acid composition, and increasing antioxidant levels in crops. This approach aims to provide sufficient calories to meet energy needs while offering all essential nutrients for sound health. Biofortifying crops consumed by the world's poor populations can significantly improve nutrient consumption in these target populations (Graham and Welch, 2001).

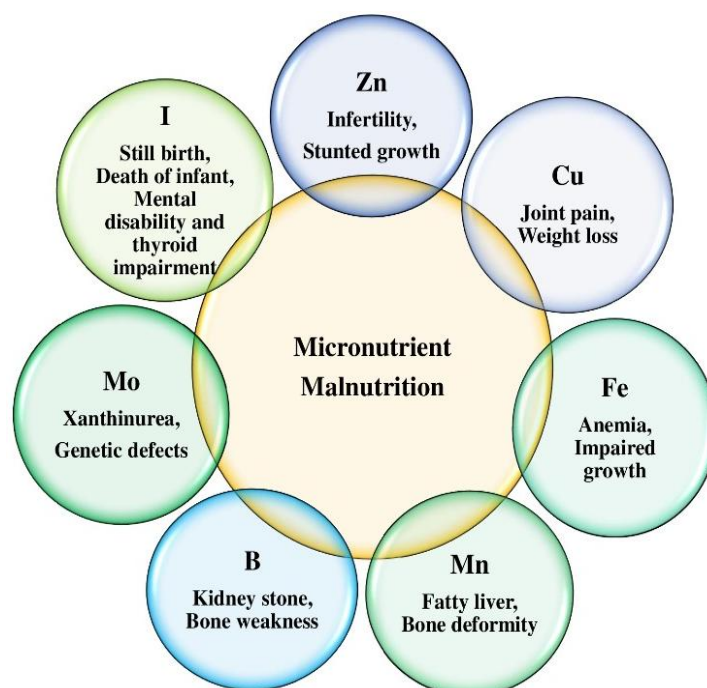


Figure 2. Effects of micronutrient malnutrition on human health (Dhaliwal et al., 2022)

Fortification, the intentional increase in essential micronutrients of staple foods, can be achieved through supplements, commercial fortification, or modifying the diet (biofortification) (Khush et al., 2012). Biofortification is considered a long-term and efficient method, particularly in regions lacking socio-economic infrastructure (Combs et al., 1997; Welch, 2002; Pfeiffer and Mc Clafferty, 2007). Biofortification, a proposed tool for alleviating malnutrition, involves enriching selected nutrients in the edible portions of crops for both human and animal consumption. This approach not only tackles hidden hunger but also enhances crop yield, demonstrating its sustainability and cost-effectiveness (Dhaliwal et al., 2022). Biofortification, achieved through agronomic methods, conventional breeding, or biotechnological tools, refers to the enhancement of nutrient content or bioavailability in staple food crops (Pérez-Massot et al., 2013). The process also contributes to better crop productivity and nutritional quality, ultimately reducing micronutrient malnutrition in consumers (Dhaliwal et al., 2022). The

consumption of biofortified staple crops is expected to lead to the measurable improvements in human health and nutrition, contributing to the fight against micronutrient malnutrition, or hidden hunger especially in regions given in *Figures 3* and *4*, facing socio-economic challenges.

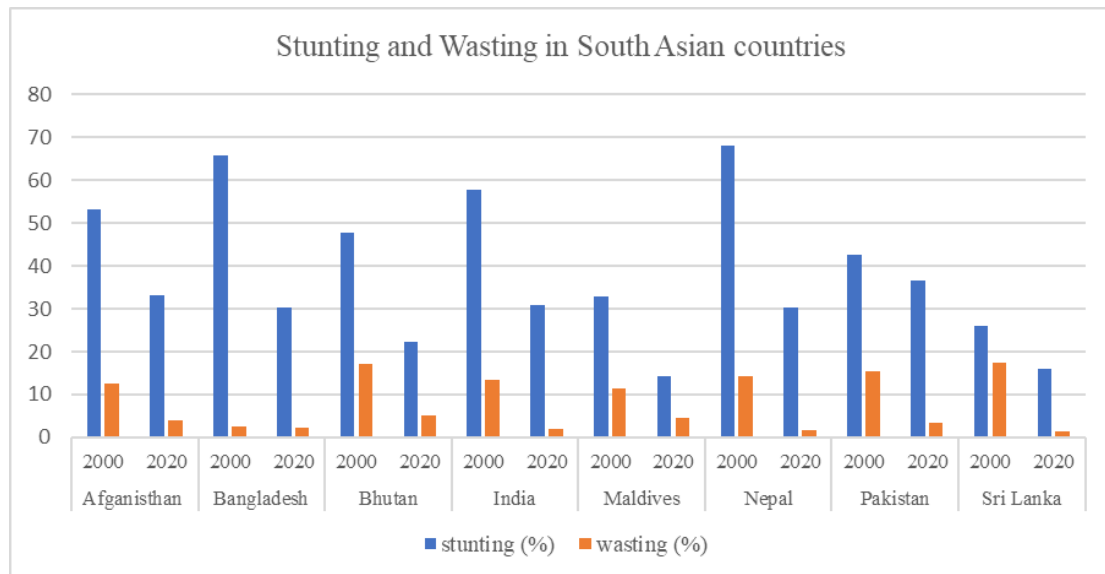


Figure 3. Nutrition indicator trends (stunting and wasting) levels in South Asian countries observed from 2000 to 2020 (Sheoran et al., 2022)

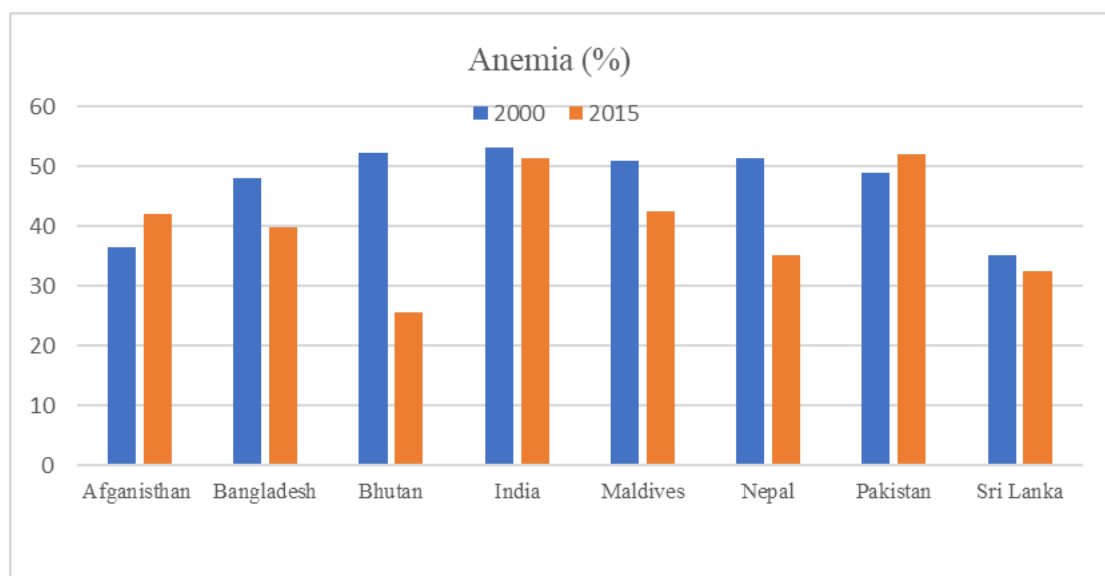


Figure 4. Nutrition indicator trends (anemia) levels in South Asian countries observed from 2000 to 2015 (Sheoran et al., 2022)

Importance of biofortifications in pulses and legumes

Pulse and legumes are consisted of excellent sources of dietary proteins, complex carbohydrates, vitamins, and minerals. Their slow-digestible carbohydrates, along with

being rich in proteins and amino acids, make them easily available and the least expensive source of proteins and micronutrients for various populations (Ghosh et al., 2019). In recent decades, there has been increased global attention to combat micronutrient malnutrition, employing strategies such as increased food production, supplementation, food fortification, and biofortification.

Biofortification efforts in pulse and legumes have gained momentum in the past decade. Various studies like, plant breeding, genetic engineering, and agronomical approaches are indicated that increasing mineral content, vitamin content, essential amino acid levels, fatty acid composition, and antioxidant levels in crops. Biofortifying crop plants can provide sufficient calories to meet energy needs while delivering essential nutrients for overall health. Moreover, focusing on biofortifying crops consumed by the impoverished populations worldwide can significantly enhance nutrient intake for this target group (Welch and Graham, 1999).

Guidelines for biofortified crops: essential criteria

The primary objective of biofortification is to cultivate staple contain higher amount, of micronutrients, aiming to mitigate micronutrient malnutrition and contribute to food security, enhanced productivity, and improved quality of life in developing countries. The success of biofortification is contingent on several key criteria mentioned in *Figure 5*.

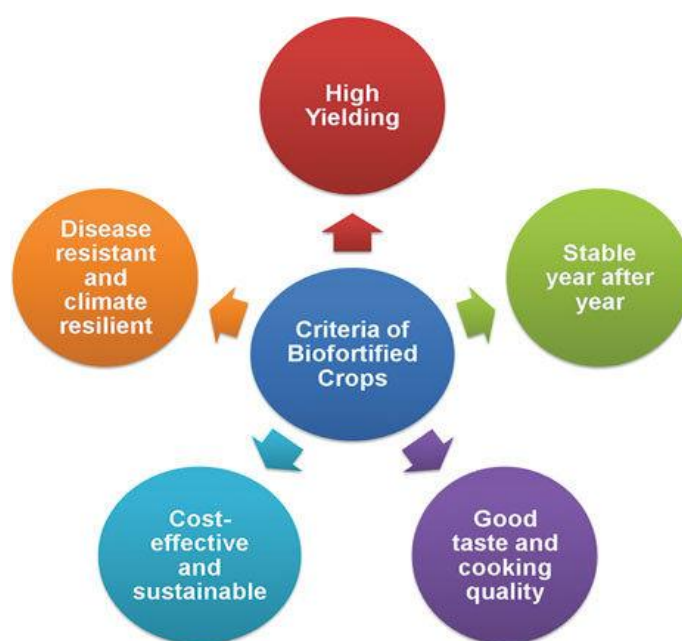


Figure 5. Criteria of biofortified crops (Singh et al., 2015)

Pulses and legumes

The term “pulse” is derived from the Latin word “puls or pultis,” which means a thick slurry. Pulses belong to the legume family and have been a part of traditional diets worldwide for thousands of years. While every pulse is a legume, not all legumes are pulses; for example, dry peas, lentils, chickpeas, and dry beans are considered pulses, whereas legumes also include soybeans, peanuts, and fresh peas (Asif et al., 2013).

Legumes are defined as pods or fruits containing seeds or dry grains and have the unique capability to fix atmospheric nitrogen in the soil. India, as the largest producer (26% of global production), consumer (27% of world consumption), and importer (14%) of pulses globally, plays a vital role in the legume market (FAO, 2020b). Despite being staple food crops for billions of people worldwide, the biofortification of legumes has not popularly utilized as an approach to alleviate hidden hunger. While there are over 1000 known legumes, only around 20 are cultivated for consumption, including cowpea, chickpea, pigeon pea, mung bean, urd bean, lentil, French bean, horse gram, field pea, soybeans, moth bean, lathyrus, etc. These pulses, when combined with other cereals, offer opportunities for use in food processing and developing various products like bakery items, bread, pasta, soaked food, snacks, soups, cereal bar fillings, and meat products (Asif et al., 2013).

The agricultural shift from focusing solely on quantity for producing nutrient-rich crops is gaining momentum, with biofortified pulses seen as having immense potential to address hidden hunger. Biofortification enhances the density of bioavailable micronutrients, minerals, and vitamins in the edible part of pulses. This agricultural strategy is considered a cost-effective means of meeting nutritional needs, particularly in developing and poor countries (Garg et al., 2018). The seed protein content and other constituents of major pulses and legume are given in Table 2.

Table 2. Seed protein content and other constituents of grain pulses and legumes

S. No	Crop	Seed protein content (%)	Oil %	Starch %	Fiber %	Sucrose %
1.	Soybean (<i>Glycine max</i>)	Up to 40	17.7–21.0	1.5	20	6.2
2.	Chickpea (<i>Cicer erietinum</i> L.)	17–22 before dehulling	15.5–28.2	44.4	9	2
3.	Common bean (<i>Phaseolous vulgaris</i>)	20–30	0.9–2.4	41.5	10	5
4.	Urd bean (<i>Vigna mungo</i>)	25–28	-	-	-	-
5.	Lentil (<i>Lens culinaris</i> Medik)	20.6 and 31.4	0.8–2	46	12	2.9
6.	Lupin (<i>Lupinus albus</i> L.)	35–44	-	-	-	-
7.	Pigeon pea (<i>Cajanus cajan</i>)	20–22	1.3–3.8	44.3	10	2.5
8.	Faba bean (<i>Vicia faba</i>)	26–41	1.1–2.5	37–45.6	7.5–13.1	0.4–2.3
9.	Mung bean (<i>Vigna radiata</i>)	20.97–31.32	1.2	45	7.0	1.1
10.	Cowpea (<i>Vigna unguiculata</i>)	14.8–25	1.3	-	-	-
11.	Pea (<i>Pisum sativum</i>)	13.7–30.7	0.6–5.5	45	12	2.1
12.	Lathyrus (<i>Lathyrus sativus</i>)	8.6–34.6	-	-	-	-

Source: Jha and Warkentin, 2020; Kumar and Pandey, 2020

Unlocking health benefits: the vital role of minerals in human body

Humans require approximately 40 known nutrients in sufficient quantities for healthy and productive lives. Essential nutrients, like sodium, calcium, potassium, magnesium, phosphorus, chlorine, and sulfur, are required in small amounts. Another class of essential nutrients, termed micronutrients, includes iron, zinc, iodine, copper, selenium, manganese, molybdenum, nickel, cobalt, and vitamin A (Prashanth et al., 2015). These nutrients collectively play major roles in human development, influencing both physical and mental aspects given in *Table 3* (White and Broadley, 2005). Various micronutrients serve as cofactors for many enzymes in the human body, regulating essential functions and metabolic processes (Welch and Graham, 2004). Agricultural products are the primary source of nutrients for humans, especially those in developing countries (Graham et al., 2001; McGuire, 1993; Schneeman, 2001). Nevertheless, diets primarily reliant on cereals such as rice, wheat, cassava, and maize frequently fail to provide adequate quantities of essential nutrients like vit- A, Fe, Zn, Ca, Mn, Cu, I, and Se to meet daily nutritional needs. Many people worldwide rely on plant-based foods that are often low in key micronutrients, leading to micronutrient malnutrition or “hidden hunger” affecting one in three people globally (FAO, 2013).

Food crops rich in nutrients, especially pulses like peas, chickpeas, lentils, common beans, and mung beans, could address these deficiencies and offer a sustainable solution to global health issues (Welch, 2002). Pulses contain plentiful source of complex carbohydrates, dietary proteins, vitamins, and minerals essential for human nutrition. The micronutrient bioavailability in pulses and their identifying promoters and inhibitors are given in *Table 4*. They are integral to traditional diets worldwide due to their richness in proteins, amino acids, and slowly digestible carbohydrates, providing an easily accessible and cost-effective source of essential nutrients. Pulse consumption has been on the rise, driven by their recognized health and environmental benefits (Curran, 2012).

The possible positive impacts of consuming legumes on human health

1. Enhanced the metabolic, immunological, and hormonal regulations
2. Anticarcinogenic effects (Colorectal, breast, prostate and endometrium cancers)
3. Inflammatory reduction effects
4. Reduced risk of cardiovascular and obesity-related diseases, and metabolic syndrome
5. Reduced cholesterol levels
6. Reduced risk of type 2 diabetes mellitus
7. Reduced risk of osteoporosis and depression (Roriz et al., 2020)

Various biofortification approaches

In the realm of biofortification, there is a predominant focus on cereals, pulses, oilseeds, vegetables, and fruits, with a primary emphasis on enhancing the nutritional content of Zn, Fe, Mg, I, Se, vitamin A, folic acid, and carotenoids (Poletti and Sautter, 2005). The endeavor for sustainable biofortification encompasses diverse approaches, such as conventional/traditional plant breeding, molecular breeding, genetic engineering, and agronomic methods, providing long-lasting solutions. Molecular and

genetic engineering, notably, are regarded as precise and accurate techniques that significantly boost the nutritional content of staple crops (Garg et al., 2018; Jha and Warkentin, 2020).

Table 3. Key micronutrients in pulses and legumes, and their role in human body

S. No	Micro-nutrients	Role in human body	Consequences	Recommended dietary allowance (RDA)	References
1.	Iron (Fe)	1. Crucial role in various metabolic processes like electron transport chain and the synthesis of deoxyribonucleic acid 2. Oxygen transporter from the lungs to the body tissues	Decrease in energy levels, dizziness, and adverse pregnancy outcomes such as premature births, low birth weight babies, delayed growth and development in infants, as well as poor cognitive skills	8 mg day ⁻¹ male and 18 mg day ⁻¹ female	Abbaspour et al., 2014; Hurrell, 1997; McDowell, 1992; WHO, 2001; Lozo et al., 2008; Allen, 2000
2.	Zinc (Zn)	1. Enhancing wound healing 2. Zinc plays a role in protecting cells from oxidative damage by quenching reactive oxygen species 3. Reduced risk of certain cancers, like pancreatic and prostate cancers	Weakened immune system, susceptibility to recurrent infections, mental health issues, and impaired growth and fertility	11 mg day ⁻¹ for adult male and 8 mg ⁻¹ day for adult female	MacDonald, 2000; Costello and Franklin, 2017; Roohani et al., 2013
3.	Selenium (Se)	1. Necessary for growth, and development 2. Providing protection against infections 3. Progression of cancer and oxidative stress	Se, is linked to various diseases, including Keshan disease, Keshin-Beck disease, and myxedematous cretinism	55 µg day ⁻¹ for both male and female	Rayman, 2005; Tinggi, 2008; Zeng and Combs, 2008; Coppinger and Diamond, 2001
4.	Iodine	Crucial component of the thyroid hormones thyroxine (T4) and triiodothyronine (T3), playing an essential role in normal growth, development, and metabolism	1. Lead to hypothyroidism, goiter, cretinism, mental retardation, reduced fertility, increased prenatal death, and infant mortality 2. During pregnancy, iodine deficiency leads to, cognitive impairment in the offspring, impacting brain development	150 µg day ⁻¹ for both male and female	Andersson et al., 2007; WHO, 2007; Skeaff, 2011; Pearce et al., 2016
5.	Carotenoids	1. Lutein and zeaxanthin have been identified as preventive agents against age-related macular degeneration 2. Reducing the risk of cardiovascular disease 3. Beta-cryptoxanthin, another carotenoid, plays a vital role in bone formation by stimulating osteoblastic bone formation and inhibiting osteoclastic bone resorption 4. Carotenoids demonstrate strong anti-cancer properties and protect cellular organelles from oxidative damage by effectively scavenging free radicals produced during diverse metabolic processes	-	-	Tanaka, 2012; Fraser and Bramley, 2004; Olmedilla, 2001; Moeller, 2000; Alves-Rodrigues and Shao, 2004; Yamaguchi, 2004; Lannone, 1998; Sujak et al., 1999
6.	Folates	1. Essential for nucleotide biosynthesis and amino acid metabolism in human body 2. Crucial for human growth and development	Increase various chronic diseases, including neural tube defects, impaired cognitive function Alzheimer's diseases, cardiovascular diseases and cancers	-	Scott, 2000; Basset, 2005; Geisel, 2003; Ramos et al., 2005; Seshadri, 2002; McCully, 2007; Choi and Friso, 2005

Table 4. *Micronutrient bioavailability in pulses: identifying promoters and inhibitors*

Factors	Nutrient	Major dietary source
Promoters		
1. Prebiotics: inulin and fructans	Fe, Zn, Ca	Lentils
2. Beta-carotene	Fe, Zn	Lentil, pea, chickpea
3. Selenium	I	Lentil, pea, chickpea
4. Organic acids: ascorbic acid	Fe, Zn	Lentils
5. Amino acids	Fe, Zn	-
Inhibitors		
1. Phytic acid	Fe, Zn, Ca	All legumes
2. Fiber	Fe, Zn	All legumes
3. Haemagglutinins	Fe, Zn	Most legumes
4. Phenolics	Fe, Zn	All legumes
5. Heavy metals	Zn	Contaminated legumes

Source: Kumar and Pandey, 2020

Conventional plant breeding/traditional plant breeding approach

Biofortification through plant breeding stands out as a sustainable method with the potential to enhance the health status of economically poor populations in worldwide (Bouis et al., 2011; Blancquaert et al., 2014). This approach has been successfully employed to address micronutrient deficiencies, including those of carotenoids, iron (Fe), and zinc (Zn) (White and Broadley, 2005; Welch and Graham, 2005). The benefits of conventional plant breeding extend not only to large populations but also to individuals residing in remote areas with limited access to commercially fortified foods (Bouis et al., 2011; Saltzman et al., 2013).

Conventional breeding requires a single investment, enabling farmers to cultivate biofortified crops over several years with nearly zero marginal cost. Ongoing expenses are minimal, and the resulting germplasms can be shared globally without negative impacts on productivity and health, gaining broad public acceptance (Nestel et al., 2006; Bouis et al., 2011; Winkler, 2011). Over time, Conventional breeding strategies have produced numerous varieties of staple crops, enhancements in essential micronutrients (Saltzman et al., 2017; Sheoran et al., 2021). Some of the recently developed biofortified varieties given in Table 5. This establishes it as the most extensively embraced and reliable method for biofortification. The successful implementation of this strategy relies on the presence of genetic diversity within crops, enabling plant breeders to effectively utilize germplasm from primary, secondary, and tertiary gene pools to identify crucial genes for the creation of biofortified varieties (Jha et al., 2020). Numerous studies have explored genetic variability for micronutrient assessment (Boy et al., 2017; Dutta et al., 2020; Govindaraj et al., 2020; White and Broadley, 2009; Garg et al., 2018).

Molecular breeding approach

The conventional process of producing a biofortified variety involves identifying and transferring desirable genes from a donor to a recipient parental line with superior agronomic characteristics, utilizing molecular breeding techniques. Progress in molecular breeding has notably improved and accelerated the creation of biofortified

varieties enriched with essential minerals, aiding in the battle against malnutrition (Pray, 2006). Molecular breeding has predominantly been utilized in staple crops like cereals, pulses, millets, fruits, and vegetables to develop biofortified varieties (Garg et al., 2018). This method reduces the number of necessary generations and enables the screening of numerous plants solely at the seedling stage. Furthermore, molecular breeding is beneficial for pinpointing recessive traits in plants, a task that conventional breeding techniques find challenging.

Table 5. Recently developed biofortified varieties through (Pure line selection) various institutes

S. No	Recently developed varieties	Conc. of nutrient	Conc. of nutrient in other popular varieties	Developed from	Year of release
1.	Lentil				
	Pusa Ageti Masoor	Rich in iron 65.0 ppm	45.0-50.0 ppm	ICAR-Indian Agricultural Research Institute, New Delhi	2017
	IPL 220	Rich in iron (73.0 ppm) and zinc (51.0 ppm)	45.0-50.0 ppm iron and 35.0-40.0 ppm zinc	ICAR-Indian Institute of Pulses Research, Kanpur	2018
2.	Soybean				
	NRC 127	Free from KTI (Kunitz Trypsin Inhibitor) in comparison to 30-45 mg/g of seed meal in popular varieties	-	Developed by ICAR-Indian Institute of Soybean Research, Indore	2018
	NRC 132	Free from lipoxygenase-2 (Lox-2)	-	Developed by ICAR-Indian Institute of Soybean Research, Indore	2020
	NRC 147	Rich in oleic acid (42.0%)	22-25%	Developed by ICAR-Indian Institute of Soybean Research, Indore	2020
	NRC 142	first double null variety for Kunitz Trypsin Inhibitor (KTI) and lipoxygenase-2 (Lox-2)	-	Developed by ICAR-Indian Institute of Soybean Research, Indore	2021
	MACSNRC 1667	Free from KTI	-	ICAR-Indian Institute of Soybean Research, Indore	2021

Source: Yadava et al., 2020

Genetic engineering/transgenic approach

Genetic engineering, an advanced biotechnological technique, encompasses the direct incorporation of genes into breeding varieties. These genes may originate from diverse organisms, such as animals and microbes, with the objective of improving mineral mobilization efficiency in the soil, lowering, anti-nutritional compounds, and augmenting the concentration of nutritional enhancer compounds like inulin (Zhu et al., 2007). Genetic engineering in biofortification serves as an alternative when desired traits are absent in available germplasm, specific micronutrients are not naturally present in crops, and/or modifications cannot be attained through conventional breeding (Mayer et al., 2008; Perez-Massot et al., 2013). Various approaches, such as overexpression, gene stacking, RNA interference (RNAi), and clustered regularly interspaced short palindromic repeats (CRISPR) or CRISPR-associated protein-9 nuclease (Cas9)-mediated genome editing, are employed to regulate the expression of the gene of interest. Innovative target-specific genome editing methods, including transcription activator-like effector nucleases (TALENs), zinc finger nucleases (ZFNs), and CRISPR/Cas9, have shown promising results in the biofortification of several crops (Ricroch et al., 2017).

Genetic biofortification is a one-time investment, addressing hidden hunger without the need for repeated purchase or addition of fortificants to the food, unlike commercial fortification. By elevating the concentration of micronutrients, in this method can concurrently address the elimination of anti-nutrients or the incorporation of promoters to improve micronutrient bioavailability, in *Table 6* represent the developed varieties through transgenic breeding (White and Broadley, 2009; Garg et al., 2018; Carvalho and Vasconcelos, 2013). While the development of transgenic crops involves a significant initial investment, it has the potential to be a sustainable approach, particularly beneficial for large populations in developing countries (White and Broadley, 2005; Hirschi, 2009; Hefferon, 2016).

Table 6. Biofortification of crops through transgenic approach

S. No	Crops	Enhanced nutrition's	Genes involved
1.	Chickpea (<i>Cicer arietinum</i>)	Iron	GmFER, NAS2
2.	Common bean (<i>Phaseolus vulgaris</i>)	Methionine	Methionine rich storage albumin
3.	Soybean (<i>Glycine max</i>)	Provitamin A	Carotene desaturase, crtB, crtW, bacterial PSY, bkt1
		Fe and zinc	Phytase
		Lysine	Aspaktokinase, dihydrodipicolinic acid
		Cysteine	Maize zein protein, O-acetyl serine sulfhydrylase
		Methionine	Cystathionin Alpha-synthase, maize zein protein

Source: Shahzad et al., 2021

Agronomic approach

Agronomic biofortification involves several approaches, such as application of mineral fertilizers in to the soil, foliar fertilization, and soil inoculation with beneficial microorganisms, to enhance the essential micronutrient content of food crops. Particularly in Asia and Africa, agronomic biofortification, considered a fast and straightforward method to supply food grains with essential micronutrients like zinc (Zn), iron (Fe), or others (Meena and Fathima, 2017). This method encompasses the use of synthetic fertilizers, organic manures, biofertilizers, and seed priming through soil or foliar application (Shivay et al., 2016). Agronomic biofortification aims to enhance the micronutrients in to the edible part of food crops through the basal or foliar application of mineral fertilizers. Commonly enriched elements include iron (Fe), selenium (Se), zinc (Zn), and iodine (I). The successful biofortification involves on the absorption of minerals from soil into plants and their accumulation in the edible parts of crops. Additionally, the bioavailability of minerals from biofortified crops plays a crucial role in determining the success of biofortification initiatives.

Much of the research on agronomic biofortification focused on Se and Zn. Se is an essential mineral play crucial role in human health. Blending or granulating Se with macronutrient fertilizers has proven to be highly effective (Cakmak, 2014). In Finland, Se concentration was increased in 15-fold due to the application of Se with NPK fertilizers in crop fields (Alfthan et al., 2015). Similarly, in Malawi, find out an increase in the Se concentration up to 88–97% in maize grain for the application of 20 g ha⁻¹ Se

fertilizer (Chilimba et al., 2012). Another study in Brazil reported a 10-fold increase in grain Se with the application of 25 g ha⁻¹ Se fertilizer (Reis et al., 2018).

Mineral fertilizer

Mineral fertilizers, composed of essential minerals in inorganic form, offer a method to enrich soil micronutrient levels and improve plant quality. Given the typically limited availability of micronutrients and minerals in soil for plant uptake, there is a need to employ micronutrient and mineral fertilizers with enhanced solubility and mobility to boost their concentration in edible plant tissues (White and Broadley, 2009). While this method is effective for fortifying plants with mineral elements.

Successful implementation of this method has been seen with elements like selenium (Se), iodine (I), and zinc (Zn) due to their good mobility in both soil and plants. For zinc (Zn), the concentration in field pea grains increased through the application of Zn fertilizer to the soil, either alone or in combination with foliar treatments. This highlights the potential effectiveness of mineral biofortification on field peas (Poblaciones and Rengel, 2016). For instance, supplementing inorganic fertilizers with sodium selenate increased the Se concentration in numerous food items like cereals, fruits, vegetables, dairy products, eggs, meat, and fish in Finland, proving to be a sustainable way to enhance Se intake in the human population (Jha et al., 2020; Alfthan et al., 2015). Similarly, successful enrichment of plants with I and Zn was achieved in China and Thailand, respectively, using inorganic fertilizers (Winkler, 2011). However, Fe fertilization faced challenges due to the low mobility of iron (Fe) in the soil (Jha et al., 2020).

Seed priming

Seed priming involves treating seeds with micronutrients through immersion in a specific concentration of a nutrient solution for a designated period. In the case of chickpea seeds, priming them in a 0.05% solution of zinc sulphate heptahydrate (ZnSO₄·7H₂O) proved highly effective, resulting in an average 19% increase in chickpea yield compared to non-primed seeds given in *Table 7* (Harris et al., 2008). Furthermore, seed priming boosted the zinc concentration in chickpea seeds by 29%, a substantial improvement. The process of priming with zinc offers benefits such as improved crop emergence, stand establishment, plant growth, yield, and nutrient concentration, as indicated by IIPR in 2014–15. While zinc priming is effective in moderately zinc-deficient soils, it may not meet the plant's zinc needs under severe deficiency. For instance, solely priming kidney beans may not be adequate to satisfy their requirements (Harris et al., 2008). Nonetheless, increasing the zinc concentration in the priming solution enhances nutrient absorption in both chickpea and lentil seeds, as demonstrated by Johnson et al. (2005).

Seed coating

Seed coating is a technique that entails applying finely ground solid or liquid substances, which may include dissolved or suspended solids, to form a uniform and continuous layer covering the seed coat (Scott, 1989). This method usually involves coating seeds sequentially with layers of adhesives, followed by finely ground nutrients after meticulous sieving, leading to the desired enhancement in seed size. Within this method, beneficial elements such as microorganisms, plant-growth

regulators, chemicals, and nutrients are affixed or administered around the seed using adhesive or viscous materials.

Table 7. Seed priming and coating for zinc and boron enrichment of pulses

Crop	Fertilizer	Rate of application and time for priming	Increase in Zn content in grain over control (%)	References
1. Seed priming				
Chickpea	ZnSO ₄ ·7H ₂ O	0.05% Zn, 6 h	29.0-36.0	Harris et al., 2008 Johnson et al., 2005 Arif et al., 2007
Chickpea		0.004 M Zn, 8 h		
Chickpea		0.05% Zn, 8 h		
Chickpea		0.075% Zn, 8 h	5.0	Arif et al., 2007
Lentil		0.004 M Zn, 12 h	11.7	Johnson et al., 2005
Cowpea		0.004% Zn, 12 h		Johnson et al., 2005
2. Seed coating				
Cowpea	ZnSO ₄ ·7H ₂ O	250 mg/kg seed	32.1	Masuthi et al., 2009
Cowpea	Borax	100 mg/kg seed	37.3	
Chickpea				

Source: Shivay et al., 2016

Seed coatings containing trace elements such as Mo, Fe, Zn, Mn, and B, have demonstrated increased effectiveness. The utilization of Zn in seed coating not only boosts Zn levels in seeds (Singh, 2007; Masuthi et al., 2009; IIPR, 2014-2015) but also enhances seed germination, plant growth, and leaf surface area. For example, coating cowpea seeds with ZnSO₄ (250 mg kg⁻¹ seed) resulted in elevated grain yield, seed weight, and overall seed production. Singh (2007) also observed improved growth, yield, and zinc concentration in soybean (*Glycine max*).

Foliar fertilization

Foliar fertilization involves the direct application of fertilizers to the leaves and can be successful when mineral elements are not immediately available in the soil or not readily translocated to edible tissues (White and Broadley, 2009; Garg et al., 2018). Various studies have demonstrated successful biofortification of pulse crops, including cowpeas, mungbeans, common beans, chickpeas, and field peas, with micronutrients like zinc (Zn), iron (Fe), and selenium (Se) through foliar application, resulting in enhanced the levels of micronutrients in the harvested grains (Table 8).

Table 8. Some of the nutrient application methods and increased nutrient concentration in crops

S. No	Crop	Application method	Application rate	Increase (%) of grain Zn, Se and Fe content
1.	Chickpea	Basal	25 kg ha ⁻¹ ZnSO ₄ ·7H ₂ O	24.9
		Foliar	0.5% (w/v) ZnSO ₄ ·7H ₂ O	35.4
		Basal and foliar	25 kg ha ⁻¹ and 0.5% (w/v) ZnSO ₄ ·7H ₂ O	39.1
2.	Soybean	Basal	80 g ha ⁻¹ Na ₂ SeO ₄	290–331
3.	Faba bean	Foliar	1 L m ⁻² Se nanoparticles (90 nm) (concentration = 100 mg L ⁻¹)	1360
			1 L m ⁻² sodium selenite (concentration = 220 mg L ⁻¹)	3799
			1 L m ⁻² sodium selenate (concentration = 240 mg L ⁻¹)	7426
4.	Chickpea	Foliar	0.5% FeSO ₄ ·7H ₂ O	-2.8
			0.5% FeO ₃ nanoparticle	0.16

Source: Teklu et al., 2023

For instance, Márquez-Quiroz et al. (2015) reported an Fe concentration (29%–32%), enhanced in cowpea seeds upon foliar application. Ali et al. (2014) observed in mung beans Fe concentration (46%) increase in with foliar Fe application. Similarly, Nandan et al. (2018) found that, the foliar application of Fe and Zn substantially increased the levels of these minerals, as well as protein, in the seeds of cowpeas and chickpeas. Shivay et al. (2015) found a correlation between Zn uptake and grain yield in chickpeas following foliar Zn application, noting that this approach was more effective than soil application. Hidoto et al. (2017) assessed various zinc (Zn) fertilization techniques for chickpeas and determined that foliar application proved to be an effective approach for Zn biofortification, resulting in higher Zn accumulation in grains compared to soil application and seed priming. Similar findings regarding foliar application of Zn fertilizer for Zn biofortification were also reported in common beans (Ibrahim and Ramadan, 2015; Ram et al., 2016; Sánchez et al., 2017) and field peas (Poblaciones and Rengel, 2016).

Se concentration enhanced in seeds of peas, common beans, chickpeas, and lentils upon foliar application of Se fertilizers (Poblaciones and Rengel, 2016; Rahman et al., 2015). Additionally, Foliar application resulted in elevated iodine (I) levels across different crops, offering a potential solution to address iodine deficiency in populations with low dietary intake of iodine (Cakmak et al., 2017).

Plant growth promoting microorganisms (PGPM)

Rhizobia, actinomycetes, mycorrhizal fungi, and diazotrophic bacteria are advantageous soil microorganisms that establish symbiotic associations with plant roots. They offer diverse protective mechanisms, including the promotion of nutrient mineralization and availability, as well as the production of plant growth hormones (FAO, 2019). Although naturally occurring in the soil, their numbers can be augmented through inoculation or agricultural management techniques. Soil microorganisms with Plant Growth Promoting (PGP) properties, such as *Enterobacter*, *Bacillus*, and *Pseudomonas*, can be employed to boost the phyto-availability of micronutrients, commonly administered as seed inoculants. These microorganisms stimulate plant growth by producing growth hormones, chitinases, antibiotics, siderophores, and inducing systemic resistance and mineralization (Mahaffee and Kloepper, 1994). PGPM plays a vital role in soil fertility and iron fortification. They chelate iron *via* the production of siderophore compounds, solubilize phosphorus, and inhibit the growth of pathogens, contributing significantly to soil health (Panhwar et al., 2012; Sreevidya et al., 2016). Numerous studies have demonstrated Fe, Se, and Zn concentrations increased through the use of microorganism inoculants *via* mycorrhizal associations. PGPM can function in various mechanisms, such as replenishing soil nutrients, enhancing nutrient availability, and facilitating improved plant nutrient uptake (Malusá and Vassilev, 2014).

PGPB promote plant growth through various direct mechanisms, such as nitrogen fixation, nutrient solubilization, production of phytohormones, siderophores, and organic acids. Indirect mechanisms include biocontrol activities, induced resistance, production of antibiotics, extracellular enzymes, and competition for rhizosphere niches (Beneduzi et al., 2012; Elshahat et al., 2016). PGPB also play a pivotal role in participating in the bioremediation of contaminated soils and managing abiotic stresses (Benidire et al., 2016; Moreira et al., 2016; Verma et al., 2019). Research has emphasized the significance of PGPB in facilitating the uptake of diverse nutrients, like as Fe, Mn, B, Zn, and Cu, achieved through the siderophore production that release of the organic acids (Ipek and Esitken, 2017). Additionally, these microorganisms

contribute to redox changes and acidification of the rhizosphere, enhancing the mobility and availability of plant nutrients (Glick, 2014; Bahadur et al., 2016; Rajkumar et al., 2010). Inoculating with PGPB has shown significant success across a range of crops, including cereals, legumes, oil crops, and vegetables (Table 9) (Karnwal, 2017; Moustaine et al., 2017; Sharifi, 2017; Kumari et al., 2018).

Table 9. Some of the bacterial genera for legume biofortification

Some bacterial genera	Crops	Contribution to biofortification
<i>Pseudomonas</i> sp. NARs1/ <i>Pseudomonas</i> sp. PGERs17 + <i>Rhizobium leguminosarum</i> -PR1	Pea, Lentil	Enhance N, P and Fe uptake
<i>Rhizobium phaseoli</i> strain 123 + <i>Pseudomonas</i> sp. LG	Common bean	Enhance N and P uptake
<i>Enterobacter</i> sp. MN17, <i>Bacillus aryabhattai</i> MDSR7 and MDSR14	Soybean, chickpea	Enhance Zn uptake
<i>Enterobacter ludwigii</i> SRI-229, <i>Acinetobacter tandoii</i> SRI-305	Pigeon pea, chickpea	Enhance Fe, Ca, Zn, Cu, and Mn uptake
<i>Streptomyces griseoflavus</i> P4 and <i>Bradyrhizobium japonicum</i> SAY3-7	Soybean	Enhance Ca, Mg, and N, P, K uptake
<i>Pseudomonas putida</i> MPJ6, <i>Pantoea dispersa</i> MPJ9	Mung bean	Enhance Fe uptake
<i>B. subtilis</i> ZM63 + <i>Bacillus aryabhattai</i> S10	Mung bean	Enhance N, P, and K uptake
<i>Serratia</i> sp. S2, <i>Serratia marcescens</i> CDP-13, <i>Pseudomonas</i> sp. RA6, <i>P. citronellis</i> (PC), <i>Symbion-K</i> (<i>Frauteria aurantia</i>)	Chickpea	Enhance micro and macro nutrient uptake

Source: Roriz et al., 2020

Advantages and disadvantages of biofortifications

Advantages	Disadvantages
Nutritional impact: Biofortification has shown positive results in increasing the nutritional content of essential micronutrient. This can have a significant impact on combating nutrient deficiencies and related health problems	Agronomic biofortification: have challenges include variability in mineral transportation and accumulation across diverse crops and varying soil compositions in different geographical locations. Additionally, this method is labor-intensive and cost-intensive, requiring repeated application of micronutrients for both the plant and soil
Health benefits: Improved nutrient levels in biofortified crops contribute to better overall health, especially in vulnerable populations such as pregnant women, infants, and young children	Conventional breeding programs: challenges may arise for specific traits, such as oil quality improvement or selenium (Se) increment, Because of restricted variability, diminished heritability, and linkage drag. Micronutrient traits, often governed by multiple genes influenced by different genetic and environmental backgrounds, pose complexities in estimation and introgression
Agricultural sustainability: Biofortification promotes sustainable agriculture by enhancing crop varieties that are already widely grown and consumed. This approach minimizes the need for additional resources cost-effective and environmentally friendly solution	Molecular breeding: indicates that only a few major staple crops, like rice, wheat, and maize, have witnessed the development of varieties through marker-assisted breeding. In tackling the constraints of traditional breeding techniques, the transgenic approach appears most favorable for broadening the genetic pool. However, it faces notable hurdles concerning regulatory procedures and widespread approval
Accessibility and affordability: The widespread cultivation of biofortified crops ensures that nutrient-rich foods are readily available and affordable for diverse communities	Acceptance and adoption: Farmers and consumers may be resistant to adopting biofortified crops due to unfamiliarity, taste differences, or skepticism about Genetically Modified Organisms (GMOs). Cultural preferences for certain crop varieties can also pose a barrier

Future perspectives of biofortification

- Need to reduce the levels of anti-nutritional compounds such as phytic acid, which inhibit the absorption of minerals like Fe, Zn, and Ca in the gut.
- To enhance biofortification programs, forthcoming research should emphasize the integration of agronomic and genetic strategies to enhance mineral transport to phloem-fed tissues.
- Multi-biofortification, the simultaneous introduction of various micronutrients into a cultivar, is considered an effective approach compared to introducing multiple biofortified crops or varieties with a single micronutrient to address diverse forms of malnutrition.

Conclusion

Biofortification of pulses and legumes is a critical advancement in combating global malnutrition. These nutrient-dense crops are staple foods in many regions, and enhancing their micronutrient content can significantly improve dietary quality. Biofortified pulses and legumes offer a sustainable, cost-effective solution to address micronutrient deficiencies and other essential nutrients. Ensuring widespread adoption and consumer acceptance, along with continued research and support, is vital for maximizing their health benefits and contributing to global food security.

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