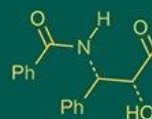


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; SP-10(6): 05-15
www.biochemjournal.com
Received: 02-04-2026
Accepted: 06-05-2026

Dhinesh

Phd Scholar, Department of
Genetics and Plant Breeding,
College of Agriculture,
Vellayani,
Thiruvananthapuram, India

M Marteena Joan

PG Scholars, Department of
Genetics and Plant Breeding,
College of Agriculture,
Vellayani,
Thiruvananthapuram, India

Adhira

PG Scholars, Department of
Genetics and Plant Breeding,
College of Agriculture,
Vellayani,
Thiruvananthapuram, India

R Praveen

Phd Scholar, Department of
Genetics and Plant Breeding,
Tamil Nadu Agricultural
University, Coimbatore, India

B Lovely

Faculty, Department of
Genetics and Plant Breeding,
College of Agriculture,
Vellayani,
Thiruvananthapuram, India

Corresponding Author:**B Lovely**

Faculty, Department of
Genetics and Plant Breeding,
College of Agriculture,
Vellayani,
Thiruvananthapuram, India

Enhancing nutritional security through bio-fortification of Major food crops

Dhinesh R, M Marteena Joan, Adhira, R Praveen and B Lovely

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i6Sa.8536>

Abstract

Bio-fortification of major food crops has emerged as a viable strategy to combat micronutrient insufficiency, widely referred to as hidden hunger. This process involves the improvement of crop nutritional quality via conventional plant breeding, agronomic interventions, or biotechnological methods. Bio-fortification seeks to elevate the dietary micronutrient status of at-risk populations by augmenting the concentrations of essential micronutrients, such as iron, zinc and vitamins in staple crops. Agronomic approaches operate by altering the cultivation environment of crops to stimulate micronutrient absorption from the rhizosphere. These approaches encompass refining soil management protocols, including targeted micronutrient fertilizer application and enhancing crop husbandry through intercropping and rotational cultivation. Plant breeding strategies concentrate on generating novel crop cultivars with superior nutritional profiles through classical genetic improvement methods. This entails hybridizing genotypes possessing favourable attributes, such as elevated nutrient density, followed by evaluation and selection of offspring exhibiting superior nutritional merit. Biotechnological strategies employ genetic manipulation to augment the nutritional attributes of crops. This encompasses deployment of transgenic technology to incorporate genes that elevate the biosynthesis of targeted micronutrients, including iron and zinc. In conclusion, bio-fortification constitutes a compelling strategy for alleviating hidden hunger and elevating the nutrient composition of food crops. Agronomic, plant breeding and biotechnological methodologies each possess inherent merits and constraints and an integrated combination of these approaches is likely to yield optimal outcomes. Continued research and development in bio-fortification will be necessary to ensure the sustainability and success of this approach.

Keywords Agronomic practices, biofortification, breeding strategies, micronutrients, nutritional safety

Introduction

Bio-fortification augments staple food crops with indispensable micronutrients and offers a sustainable intervention to counteract widespread micronutrient deficiencies (Bouis and Saltzman, 2017). Bio-fortified crops provide nutrient-rich options and are a cost-effective investment (Pfeiffer and McClafferty, 2007). Agronomic bio-fortification improves plant growth and nutrient profiles and plays a vital role in fighting poverty (Hornick, 1992) ^[36]. However, further research is necessary to enhance crop yield and nutritional content. Cereals, such as rice, wheat, maize and millet, hold historical significance and contribute to energy, carbohydrates, protein and other nutrients (Ram and Mishra, 2010). Despite their nutritional importance, cereals frequently exhibit micronutrient insufficiencies, which are particularly pronounced in low-income nations. Bio-fortification of cereal crops using molecular approaches like marker-assisted breeding, genetic engineering and gene editing has successfully developed nutrient-enriched rice and maize varieties. Molecular bio-fortification has become an indispensable instrument in tackling undernutrition and realizing food security targets by 2030 (FAO, 2020) ^[24]. Rapid population growth and limited natural resources have created a burden on the world impacting food security and nutritional quality. Bio-fortification, a genetic approach to breeding nutrient-enriched crops, offers a cost-effective solution to hidden hunger. By leveraging genetic and genomic tools, such as high-throughput phenotyping and marker-assisted breeding, bio-fortification aims to combat malnutrition and achieve sustainable food security goals. Integration of genomics and computational tools enables precise trait selection, paving way for a healthier and more sustainable future (Saltzman *et al.*, 2013; Mayer *et al.*, 2008; Beyer *et al.*, 2002).

Nutrition Silent Crisis: Unveiling the Global Scarcity of Vital Micronutrients

Insufficient nutrient availability for plants affects their food synthesis system, posing a severe issue for humans who rely on crops for sustenance. Micronutrient inadequacies in staple cereals such as wheat and rice have escalated into a worldwide public health emergency, affecting more than 2 billion individuals. This leads to compromised physical and mental development, increased disease susceptibility and issues like mental health disorders, blindness and reduced productivity. In the global landscape of malnutrition, developing countries emerge as the primary arena, out of a staggering 792.5 million undernourished individuals worldwide, a substantial 780 million can be attributed to developing countries (McGuire, 2015). A considerable fraction of the global population is burdened by multiple nutritional deficiencies, encompassing iron (Fe) deficiency (60%), zinc (Zn) deficiency (30%), iodine (I) deficiency, along with communal deficiencies of calcium (Ca), copper (Cu) and magnesium (Mg), while 15% are affected by selenium (Se) deficiency (Kaur *et al.*, 2020) [40]. Approximately half of the global population is afflicted by micronutrient malnutrition, particularly attributable to inadequate dietary consumption of selenium (Se), zinc (Zn) and iodine (I) owing to restricted dietary diversity (Mao *et al.*, 2014). Crops represent the principal repository of essential nutrients, delivering sustenance and metabolic energy to the human population. Cereal crops, constituting three main dietary staples, function as the predominant food source for roughly four billion people, supplying approximately 60% of the plant-derived caloric intake of humanity (Frison, 2016) [6].

I. Enhancing crop nutrition through effective practices of agronomic bio-fortification

India is a country with highly diverse climatic and soil conditions with high heterogeneity in soil spread across different states (Table 1). Agronomic bio-fortification, realized through economically efficient and temporally optimized agronomic interventions, enhances crop nutritional composition and physiological status (Hefferon, 2015) [35]. By deploying physical measures, such as judicious fertilizer application, rotational cropping and soil fertility management, the micronutrient quality of crops and rhizospheric soils can be substantially improved (Cakmak and Kutman, 2018) [13]. Strategies such as balanced NPK and S fertilizer application, combined with agrotechnical measures including tillage, organic amendment and soil amelioration, contribute to augmented crop productivity and elevated nutrient content (De Valença *et al.*, 2017) [19]. Micronutrient supplementation, encompassing zinc fertilization and foliar spray techniques, further improves crop nutritive quality (Lawson *et al.*, 2015). By combining micronutrient management strategies with improved high-yielding cultivars, agronomic bio-fortification can substantially elevate the nutrient density and productivity of crops, thereby promoting human health (Zuo *et al.*, 2004). Agronomic bio-fortification functions as a pivotal mechanism to augment the dietary intake of targeted minerals directly from biofortified crop sources, particularly

benefiting nutritionally vulnerable communities (Hefferon, 2015) [35]. Organizations like HarvestPlus, Biofort, CIMMYT and IITA play a significant role in developing and disseminating bio-fortified crops at the global level (Cakmak and Kutman, 2018) [13]. By guaranteeing the presence of essential micronutrients at concentrations below 1 mg/day, agronomic bio-fortification elevates food quality and contributes to improved human health outcomes (Cakmak 2010). The incorporation of NPK fertilizers, organic amendments and high-yielding cultivars reinforces the effectiveness of agronomic bio-fortification, culminating in elevated nutritional value and crop yield (De Valença *et al.*, 2017) [19]. This approach paves the way for producing cost-effective bio-fortified crops and making vital microelements more accessible for human consumption.

Table 1: The spectrum of micronutrient scarcity in the soils of India (Shukla *et al.*, 2021)

States	Per cent deficient sample (PSD)					
	Zn	Cu	Fe	Mn	B	Mo
Andhra Pradesh	46.8	<1.0	2.8	1.2	53.0	49.0
Assam	34.0	-	2.0	20.0	17.0	-
Bihar	54.0	3.0	6.0	2.0	1.0	1.0
Gujarat	23.9	4.0	8.0	4.0	57.0	8.0
Haryana	60.5	2.0	20.0	4.0	-	-
Himachal Pradesh	42.0	0.0	27.0	5.0	32	5.0
Karnataka	72.8	5.0	35.0	17.0	53	49
Kerala	34.0	3.0	<0.1	0.2	17	-
Madhya Pradesh	44.2	<1.0	7.3	0.2	49	30
Maharashtra	83.0	0.0	24.0	0.4	-	-
Meghalaya	57.0	2.0	0.0	23.0	-	-
Orissa	52.5	<1.0	0.0	0.6	-	-
Pondicherry	8.0	4.0	2.0	3.0	-	-
Punjab	46.1	1.1	14.0	2.3	-	-
Rajasthan	21.0	-	-	-	14	-
Tamil Nadu	58.6	6.0	17.0	6.0	68.0	-
Uttar Pradesh	45.7	1.0	6.0	3.0	45	12
West Bengal	36.0	0.0	0.0	3.0	49	30

Bio-fortification via agronomic measures, involves various techniques to enhance the nutritional content of crops

Fertilizer use becomes far more effective when it is applied in a targeted manner, taking into account the specific needs of different crops as well as their growth stages, rather than following a uniform approach. In addition, fertilizers can be coated with material like zinc to enable a slow and controlled release of nutrients, which helps plants absorb them more efficiently over time. Improving soil health is equally important, especially in problematic soils such as acidic or salt-affected ones, for example lime can be added to neutralize acidity, while gypsum is useful in managing salinity issues. The use of bio-fertilizers, including beneficial micro-organisms like *Rhizobium*, *Azotobacter*, and *Azospirillum*, further supports nutrient availability and enhances plant growth, while green manures, often grown as cover crops, contribute organic matter and improve overall soil fertility. At the same time, maintaining a proper balance of essential nutrients such as nitrogen, phosphorus, potassium, and sulphur is crucial for optimal plant development and efficient mineral uptake, making balanced fertilization a key agronomic practice. Crop rotation also plays a vital role, as alternating different crops on the same land helps break pest and disease cycles, reduces nutrient depletion, and restores soil health due to the varied nutrient

demands of different crops. Finally, proper soil moisture management along with appropriate tillage practices ensures that nutrients remain available in the soil and are taken up effectively by plants, ensuring better growth and productivity (Deshmukh *et al.*, 2023) ^[20]. This method

significantly enhance micronutrient accumulation across diverse crops, with the extent of improvement varying depending on the application method, crop type and nutrient involved (Table 2).

Table 2: Bio-fortified crop varieties through agronomic biofortification:

Application technique	Crop	Nutrient	Biofortification Level (%)	Reference
Soil application	Fodder	Zn	13	Grujcic <i>et al.</i> , 2021 ^[32]
	Rice	Zn	12	Tariq <i>et al.</i> , 2007
	Wheat	Zn	32	Chattha <i>et al.</i> , 2017 ^[14]
	Corn	Fe	66	Saleem <i>et al.</i> , 2016
Foliar application	Rice	Zn	35	Ram <i>et al.</i> , 2021
	Wheat	Zn	21	Ariz <i>et al.</i> , 2019
	Corn	Fe	52	Saleem <i>et al.</i> , 2016
	Eggplant	Cu	59	Bana <i>et al.</i> , 2021 ^[7]
Soilless cultivation	Lettuce	Fe	20-54	Giordano <i>et al.</i> , 2019 ^[29]
	Red Cabbage	Zn	75 to 281	Di <i>et al.</i> , 2019

Constraints of agronomic bio-fortification

The efficacy of agronomic bio-fortification is substantially dependent on the mode of fertilizer administration. Distinct fertilizer delivery methods can differentially influence micronutrient concentrations in grain tissue. Excessive micronutrient fertilizer input may suppress crop productivity and adversely impact plant physiology. Notably, micronutrient fertilizer application can enhance grain yield without proportionately elevating the micronutrient content. Among the various delivery approaches, foliar application is generally regarded as the most effective modality. Nevertheless, in cereal crops, precise timing of foliar fertilizer application relative to developmental stage is critical to attain desirable outcomes, as off-stage application confers limited benefit.

II. Advancing bio-fortification in crops through Molecular Techniques

Bio-fortification strategies targeting specific nutrient traits in cereal crops rely on molecular methodologies. These include whole-genome sequencing of crop species, genomic-level modifications such as sequence editing or targeted mutagenesis and transcriptomic analysis of the modified genotype. This pipeline identifies promising variants exhibiting transgressive segregation for the target nutritional trait and their phenotypic stability is subsequently evaluated. Validation at both the molecular and phenotypic levels is conducted to confirm consistent expression of the target trait.

A. Genomic approaches

1. Genome Sequencing/Resequencing

Whole-genome sequencing of diverse crop and non-crop plant species has uncovered novel genetic variations in nutritional traits. Such variants can be introgressed into elite crop backgrounds using marker-assisted breeding platforms. Rice was the pioneering crop to undergo complete genome sequencing under the International Rice Genome Sequencing Project in 2005. Subsequently, more than 3000 distinct rice accessions have been resequenced globally, facilitating the identification of unique genes and QTLs (quantitative trait loci) associated with micronutrient accumulation. This has markedly broadened our understanding of micronutrient variability across crop genotypes.

2. QTL mapping

Liu (1997) illustrated that QTLs are genetic regions that consist of multiple genes with small individual effects, which collectively influence quantitative traits. Unlike qualitative traits, the variation in quantitative traits is continuous rather than discrete. Gande *et al.* (2014) ^[27] further highlighted the importance of QTLs concerning the functional aspects of micronutrient uptake, transport and accumulation in grains. They emphasized the coordinated role of specific genes/QTLs under conditions of deficient or excessive micronutrient levels, which can be utilized to enhance the concentration of respective micronutrients. Sequencing and analysing these QTLs allow for techniques like knock-out gene or overexpression analysis, which provide valuable information about the genes or sequences of interest. Once the QTLs are sequenced, the information can be used to design functional markers. This facilitates QTL mapping through fine mapping or their utilization in breeding programs, such as marker-assisted selection (MAS) to improve the trait.

3. Micro RNA discovery

MicroRNAs (miRNAs) were identified relatively recently and have since attracted considerable scientific attention among plant molecular biologists. Systematic investigations have progressively elucidated their biological functions, expression patterns and structural characteristics, particularly with respect to metabolic regulation and post-transcriptional gene silencing. Unlike genomic DNA, miRNAs lack exonic sequences and do not encode functional proteins. Rather, they operate primarily as regulatory intermediaries, facilitating post-transcriptional mRNA cleavage and translational repression in gene expression modulation. Kehr (2013) and Fischer *et al.* (2013) ^[25] have reported the involvement of miRNAs in nutrient uptake and transport. Agarwal *et al.* (2015) ^[1], provide evidence of miRNAs playing a potential role in modulating zinc and iron homeostasis and the associated signal transduction cascade under micronutrient-deficient conditions in rice.

4. Epigenetics

Epigenetics refers to the collective impact on gene expression resulting from cellular components such as

chromatin structure, histone modification and DNA methylation. In their study on stress signalling and pathways, Chinnusamy and Zhu (2009) ^[16] highlighted how gene expression can be regulated through epigenetic modifications in DNA sequences upon exposure to stress signals. This regulation plays a role in developing stress tolerance or resistance mechanisms in crop plants. Duan *et al.* (2015) demonstrated that DNA methylation patterns are influenced by iron (Fe), indicating the involvement of

epigenetic regulation. The emergence of molecular biology tools and techniques has unveiled the underlying mechanisms of epigenetic regulation for stress resistance and tolerance, offering new opportunities for breeding crops that can withstand various abiotic and biotic stresses. While conventional breeding has limited success in combating stress, exploiting the concept of epigenetic regulation has proven beneficial in mitigating crop loss and increasing yield potential.

Table 3: List of different biofortified crop varieties released through molecular breeding by various institutes.

Crop	Nutrient	Variety	Level of Target Nutrient	Breeding Approach	Country	References
Rice	Fe and Zn	BRR1 Dhan 62, BRR1 Dhan 72, BRR1 Dhan 64	18–25 mg kg ⁻¹	Conventional Breeding	Bangladesh	CIAT, HarvestPlus
		Binadhan-20	20–31 mg/L Fe	MABB	Bangladesh	Noor <i>et al.</i> , 2019
		IR68144-3B-2-2-3	21 mg/kg Fe	Selection	India	Gregorio <i>et al.</i> , 2000 ^[31]
		Jalmagna	21 mg/kg Fe	Selection	India	Gregorio <i>et al.</i> , 2000 ^[31]
	Zn	DRR Dhan 49, DRR Dhan 48, DRR Dhan 45	22.6–25.2 ppm	Backcross and Pedigree	India	IIRR, India
	Protein	CR Dhan 311 (Mukul), CR Dhan 315	10.2%	Backcross followed by pedigree selection	India	NRRI, India
Wheat	Zn	BHU 1, BHU 3, BHU 5, BHU 6, BHU 17, BHU 18, Zinc Shakti (Chitra)	40–45 ppm	Conventional methods	India	CIAT, CIMMYT, Harvest Plus
		PBW1Zn	40.6 ppm	Conventional	India	PAU, India
	Protein and Fe	Pusa Ujala (HI 1605)	43 ppm Fe, 35 ppm Zn, 13% protein	Pure line selection	India	IARI India
	Protein	PBW 752	12.5% protein	Conventional	India	PAU, India
Maize	Lysine and tryptophan	Pusa HM4 Improved, Pusa HM8 Improved, Pusa HM9 Improved	3.62% lysine 0.91% tryptophan	MAS	India	CIMMYT; VPKAS, India; IARI, India
	Provitamin A, lysine, and tryptophan	Pusa Vivek QPM9 Pusa HQPM 5 Pusa HQPM 7	8.15 ppm provitamin 2.67% lysine 0.74% tryptophan	MABB	India	IARI, India
Pearl Millet	Fe and Zn	HHB 299 AHB 1269Fe ABV 04 AHB 1200Fe Hybrid ICMH 1201 (Shakti-1201)	73.0 ppm Fe, 41.0 ppm Zn 91.0 ppm Fe, 43.0 ppm Zn 70 ppm Fe, 63 ppm Zn 73.0 ppm 83.0 ppm	Conventional breeding	India	HAU, VNMKV, India with ICRISAT; MPKV, India
Sorghum	Fe	12KNICSV (Deko)-188 12KNICSV -22 (Zabuwa)	128.99 ppm Fe	Conventional breeding	Nigeria	ICRISAT, HarvestPlus
Finger Millet	Fe	VR 929 (Vegavathi)	131.8 mg/kg Fe, 33.2 mg/kg Zn	Pedigree selection	India	ANGRAU, India
Little Millet	Fe and Zn	CLMV1	59.0 ppm Fe,	Pedigree selection	India	IIMR, India

The Table 3 provides valuable insights into the efforts to enhance the nutritional content of important crops worldwide. It showcases the levels of essential nutrients such as iron, zinc and protein in varieties of rice, wheat, maize, pearl millet, sorghum, finger millet and little millet. The data reflects the application of various breeding approaches, including conventional methods and modern techniques, aimed at addressing nutritional deficiencies and ensuring food security for global populations.

B. Genomic engineering

1. Mutation Breeding

The term "mutation" refers to heritable changes in the genetic instructions encoded in DNA. These changes can be either harmful or beneficial. Beneficial mutations are used in mutation breeding, where mutants are created either spontaneously, naturally, or artificially through exposure to physical mutagens like radiation or chemical mutagens such as base analogues, alkylating agents and acridine dyes. These mutants introduce new genetic variations that do not occur naturally, allowing for the exploitation of functional variability in breeding programs to improve specific traits. Numerous mutants have been documented in various crops, including soybean, cotton and rice, for traits such as high yield, early maturity, disease resistance, grain quality and abiotic stress resistance. Therefore, mutation breeding has the potential to shape nutritional breeding, among other

traits. Wang *et al.* (2017) studied a mutant rice variety called "Lilizhi" which exhibited up to a 35% increase in zinc content compared to the original rice genotypes by enhancing the transport of stored zinc from the roots to the grains.

2. Molecular breeding

This method is widely used for bio-fortification in cereal crops such as wheat, rice, maize and millet, targeting traits like iron (Fe), zinc (Zn), provitamin A and grain protein content. It involves identifying the specific gene or allele associated with the desired trait and utilizing polymorphic DNA markers like SSRs and SNPs for background selection. This approach reduces time and effort compared to conventional methods. It includes different stages such as foreground selection, where the marker is located near the target gene, recombinant selection and background selection to retain as much of the recurrent genome as possible. Alternatively, genomic selection (GS) is a modern approach that estimates the genotypic breeding value of individuals. GS is particularly suitable for transferring complex quantitative trait loci (QTLs) compared to marker-assisted selection (MAS). The key requirements for GS have closely linked markers to the target trait, polymorphic DNA markers and specific QTLs or genes associated with the trait of interest. Table 4 lists the various works done in biofortified varieties through conventional breeding methods.

Table 4: Biofortified crop varieties developed through conventional or molecular breeding approaches

Crop	Targeted Nutrient	Variety	Level of Target Nutrient	Breeding Approach	Reference
Rice	Fe and Zn	Binadhan-20	20-31 mg/L Fe	MABB	Noor <i>et al.</i> , 2019
		IR68144-3B-2-2-3	21 mg/Kg Fe	Selection	Gregorio <i>et al.</i> , 2000 ^[31]
Wheat	Fe, Zn and protein	Pusa Tejas	42.1 ppm Fe, 42.8 ppm Zn, 12% protein	Pure line selection	Ambati <i>et al.</i> , 2019 ^[4]
Maize	Protein	Vivek QPM 9	41% higher tryptophan, 30% higher lysine	MAS	Gupta <i>et al.</i> , 2013 ^[33]
	Protein	Pusa HM-9 Improved	Improved lysine content (48-74%)	MAS	Yadava <i>et al.</i> , 2017
	Protein	HQPM-1,4,5,7	Increased proA (9.0-12.9 ppm)	MAS	Zunjare <i>et al.</i> , 2018
	Vitamins	QPMparental lines	Improved vitamin E using MAS	MAS	Das <i>et al.</i> , 2018 ^[18]
	Fe and Zn	QPM hybrids	Enhanced Fe and Zn concentration	MAS	Pandey <i>et al.</i> , 2015
Pearl millet	Fe	GB 8735	53.60 mg	Conventional breeding	Anderson <i>et al.</i> , 2017 ^[5]
	Fe	ICTP 8203	80% increased Fe content	MAS	Rai <i>et al.</i> , 2014
	Fe	ICTP 8203-Fe	Released as "Dhanashakti" for cultivation with >80% Fe target	MAS	Singh and Uddeen, 2016
	Fe	ICMH-1201	High Fe content (+28 ppm) and 38% higher grain yield	MAS	Govindaraj <i>et al.</i> , 2016 ^[30]

Limitations in enhancing nutritional content through molecular approaches

1. Presence of Nutrient Inhibitors: The presence of anti-nutrients in millets, such as phytic acid, can hinder the absorption of essential minerals like zinc, calcium and iron. These anti-nutrients, while having beneficial functions, can also interfere with the expression and availability of other micronutrients.

2. Trade-off between yield and micronutrient improvement

Breeding lines and cultivars of pearl millet that have been selected for improved yield may exhibit a negative or

insignificant correlation with micronutrient content. This is because the focus of selection was primarily on yield, rather than specific micronutrients. However, there is a positive relationship between iron and zinc, so breeding for high-iron pearl millet may inadvertently lead to increased zinc content as well.

3. Challenges in Hybridization: Artificial hybridization is necessary for developing mapping populations, but it can be challenging in self-pollinated millets like foxtail millet, finger millet and Kodo millet. These millets have small flowers, making emasculation and pollination procedures difficult and time-consuming.

4. Consideration of micronutrient bioavailability

while gene/QTLs for nutrient deficiencies can enhance their expression, it is crucial to also consider the bioavailability of the micronutrients. Simply increasing their expression does not guarantee improved nutrient absorption and utilization.

5. Complex Interactions with the Environment

The interaction between QTLs and environmental factors, along with the additive gene action of multiple loci, complicates marker-assisted selection. The influence of climate and the complex genetic architecture pose challenges in accurately predicting and selecting desired traits.

III. Bio-fortification in crops through transgenic approach

Transgenic methodologies represent a feasible alternative for generating bio-fortified crops particularly when inherent genetic variability for nutritional traits is limited within crop germplasm. These methods facilitate the transfer and stable expression of beneficial genes across taxonomically distant plant species without evolutionary constraints. Furthermore, transgenic approaches become indispensable when a particular micronutrient is naturally absent in a crop, as they permit direct fortification with the target nutrient. A thorough comprehension of gene function and the capacity to engineer plant metabolic pathways have been pivotal in transgenic crop development. By introducing heterologous genes from diverse sources, it is feasible to elevate micronutrient concentrations and their bioavailability in edible tissues while simultaneously suppressing anti-nutritional factors that impede mineral absorption. Transgenic techniques additionally enable targeted redistribution of micronutrients among plant organs, enrichment of their levels in consumable portions and optimization of biosynthetic pathways. When certain crop species are inherently deficient in a specific micronutrient, transgenic interventions emerge as the only viable strategy to enhance the nutritional profile of these crops (Perez-Massot *et al.*, 2013). Additionally, the absence of phylogenetic barriers in genetic engineering permits the design and deployment of synthetic gene constructs. These innovations in transgenic technology hold considerable promise for mitigating micronutrient deficiencies, particularly among underprivileged populations in developing nations, by increasing the levels of vitamins, minerals, essential amino acids and essential fatty acids in food crops (Garg *et al.*, 2018) ^[28].

1. Transgenics:

Due to the limited success and complexity of achieving nutritional targets through conventional breeding, alternative approaches that are target-specific have gained attention. One such option is transgenics, although it raises ethical concerns, it has yielded significant achievements. Several researchers have reported the increase in storage iron (Fe) in rice seeds ranging from 1.5 to 3.7 fold by introducing the ferritin gene (OsGluB1 proSoya-ferH1) through transgenic technology in various studies (Paul *et al.*, 2012). Overexpression of NAS genes (OsActin1 Pro-HvNAS1 and 35S Pro-HvNAS1) has resulted in two- to four-fold enhancement of Fe translocation, as reported by different researchers (Johnson *et al.*, 2011) ^[38]. Similarly, the overexpression of "OsNAS2" and "OsZIP4" genes has been

shown to enhance Iron and zinc (Zn) levels (Ishimaru *et al.*, 2007). Wirth *et al.* (2009) reported a six-fold increase in NAS and a 1.3-1.5-fold increase in ferritin transgenes. Transgenic approaches offer an efficient method to improve complex traits within a shorter timeframe and with high precision. However, there is a need for a legitimate framework to address the use of transgenes in nutritional improvement programs to address potential complications.

2. Genome editing:

A genome encompasses all the DNA, genes and information necessary for an organism development and functioning. Genome editing involves manipulating gene sequences to understand their roles. To achieve this, engineered nucleases act as molecular scissors and cut specific DNA segments. These cut sequences can then be inserted, replaced, or removed as desired. Genome editing employs different types of nucleases, including TALENs (Transcription activator-like effector nucleases), ZFN (Zinc finger nuclease) and CRISPR (Clustered regularly interspaced short palindromic repeats). Each nuclease has unique components that enable precise cutting at targeted locations. This process, with its precision and specificity, facilitates the creation of mutations or substitutions in cells. Genome editing has various applications, such as gene transfer, modifying gene expression, enhancing stress resistance, editing multiple genes and silencing genes. It is a powerful tool for controlled modification of gene sequences, including promoters and regulatory regions. Researchers widely employ this concept to investigate and improve various traits. In the context of bio-fortification, both transgenics and genome editing techniques hold immense promise in revolutionizing crop improvement and addressing nutritional challenges on a global scale. While transgenics allow the incorporation of desired genes to enhance nutrient content and distribution, genome editing offers the precision to manipulate gene sequences and optimize crop traits for improved nutritional outcomes. Through these innovative approaches, the quest for more nutritious and resilient crops takes a significant stride forward. However, careful consideration of ethical and regulatory aspects remains essential to ensure the responsible and sustainable use of these powerful tools in agriculture and food security efforts.

Various types of bio-fortification through transgenic approach

i. CRISPR mediated bio-fortification

CRISPR-mediated bio-fortification represents a transformative genome editing platform that has fundamentally reshaped the domains of agriculture and human nutrition. This advanced molecular technology equips researchers with the capability to precisely engineer the genomic sequences of organisms, including crop plants, at unprecedented resolution. By selectively targeting and modifying genes governing nutritional traits in crops, this approach unveils new avenues for substantially elevating concentrations of vital vitamins and minerals in food commodities (Table 5). A principal advantage of CRISPR-mediated bio-fortification is its capacity to execute highly efficient and site-specific genomic modifications. This precision guarantees that exclusively the intended genetic loci are altered, yielding crop varieties with markedly enhanced nutritional compositions. Furthermore, this methodology differentiates itself from conventional

transgenic approaches by exploiting endogenous genetic variability within a given species. This characteristic renders it a more precise and broadly acceptable technology within the scientific and regulatory communities. With CRISPR-mediated bio-fortification, prospects for combating undernutrition and strengthening food security are substantially enhanced. By engineering crops with elevated

concentrations of critical nutrients, investigators endeavour to beneficially influence global public health and reduce nutritional deficiencies in susceptible populations. As this technology progressively matures, its significance in determining the trajectory of agriculture and nutritional science is expected to grow considerably.

Table 5: List of crops biofortified through CRISPR mediated bio-fortification

Crops	Crispr-cas systems	Targeted genes	Function	Reference
Rice	CRISPR-Cas9	SBEI and SBEIIb	Generation of targeted mutagenesis in Starch branching enzymes SBEI and SBEIIb to create high-amylose rice	Sun <i>et al.</i> (2017)
Wheat	CRISPR-Cas9	α -gliadin genes	Two sgRNAs target the α -gliadin gene for α -gliadin reduction, which results in low-gluten meals	Sánchez-León <i>et al.</i> (2018)
Sorghum	CRISPR-Cas9	k1C gene family	Knockdown of genes k1C increased digestibility and protein quality	Li X. <i>et al.</i> (2018)
Peanut	CRISPR-Cas9	FAD2A and FAD2B	Knockdown of genes FAD2A and FAD2B increased oleic acid content	Yuan <i>et al.</i> (2019)
Soybean	CRISPR-Cas9	FAD2-1A and FAD2-1B	Knock-down of genes FAD2-1A and FAD2-1B altered high oleic acid and low linoleic acid content	Haun <i>et al.</i> (2014) ^[34]
Rapeseed	CRISPR-Cas9	FAD2	Knockdown of FAD2 increased oleic acid content and decreased linoleic and linolenic acid contents	Okuzaki <i>et al.</i> (2018)
Cassava	CRISPR-Cas9	GBSSI	Knockdown of GBSSI decreased amylose contents	Bull <i>et al.</i> (2018) ^[11]
Potato	CRISPR-Cas9 and CBE	StGBSS	Loss of function of the StGBSSI protein is related to impaired amylase biosynthesis	Veillet <i>et al.</i> (2019)
Sweet Potato	CRISPR-Cas9	GBSSI	Knockdown of GBSSI decreased the decreased amylase content	Wang <i>et al.</i> (2019)

ii. Gene overexpression

Gene overexpression is an advanced molecular strategy deployed in modern agricultural biotechnology to amplify beneficial agronomic traits in crops. This approach entails the deliberate selection of target genes governing desirable traits, such as elevated nutritional content and driving their constitutive overexpression. To accomplish this, specialized regulatory promoters are utilized to upregulate the transcriptional activity of selected genes within the crop genome *via* recombinant DNA technology, the identified genes and associated promoters are stably integrated into the crop genome, enabling their consistent expression. As a consequence, the overexpression of these target genes results in a substantial accumulation of specific nutrients, generating crops with superior nutritional value and enhanced capacity to positively influence human wellbeing. Beyond its immediate nutritional benefits, gene

overexpression holds the potential to elevate crop functionality and nutrient accessibility. By using this approach, crops can be tailored to possess heightened bioavailability and improved functionality of certain nutrients (Table 6). This multifaceted technique offers a promising avenue to tackle global nutritional challenges and elevate the nutritional quality of staple foods. The application of gene overexpression represents a dynamic and transformative advancement in agriculture, as it empowers researchers and farmers to create crops that align with the nutritional demands of a burgeoning world population. As scientific knowledge and technology continue to progress, gene overexpression emerges as an exciting and promising frontier for revolutionizing crop improvement and contributing to a more sustainable and health-conscious food system.

Table 6: List of crops biofortified through gene over-expression

Gene Overexpression	Observed Effects	Reference
OsHMA3	Reduces cadmium concentrations in shoots and grains	Lee <i>et al.</i> (2007)
AtZIP1	Increases iron levels in barley roots	Ramesh <i>et al.</i> (2004)
Phytase	Reduces phytate concentrations in seeds of various crops	Chen <i>et al.</i> (2008) ^[15]
Endogenous NAS genes	Increases nicotinamide, iron and zinc concentrations in rice	Wirth <i>et al.</i> (2009)
HGGT and HPT	Increases in tocochromanol content in tobacco, Arabidopsis and maize	Tanaka <i>et al.</i> (2015)

iii. Foreign gene transfer

Foreign gene transfer constitutes an innovative approach in modern agricultural biotechnology, directed towards augmenting the nutritional composition and mineral density of crop species. This procedure involves the identification of heterologous genes from varied biological sources, selected for their demonstrated ability to confer nutritional enhancements (Table 7). Through sophisticated recombinant genetic engineering, these exogenous genes are stably

incorporated into the recipient crop genome, resulting in the generation of transgenic crop varieties.

The introduction of exogenous genes into crop genomes expands the biosynthetic repertoire, enabling the production of novel enzymes or functional proteins that substantially enhance the plant capacity to synthesize and accumulate essential nutrients. Consequently, transgenic crops acquire unique biochemical attributes not inherent to their wild-type counterparts. This sophisticated biotechnological approach holds significant potential in tackling global food security

concerns and fulfilling the nutritional demands of a rapidly expanding world population. While the application of foreign gene transfer has generated debate on ethical and

regulatory grounds, its potential to revolutionize crop enhancement and stimulate innovation in agriculture continues to drive extensive research and exploration.

Table 7 Biofortification through alien gene transfer in various crops

Targeted Crop	Targeted Nutrients	Gene	Donor Organism or Technique	References
Rice	Fe	AtIRT1, AtNAS1, PvFER	<i>Arabidopsis</i> , common bean	Boonyaves <i>et al.</i> , 2016 ^[9]
	Fe, Zn, B-carotene	AtNAS1, PvFERRITIN, CRTI, ZmPSY	<i>Arabidopsis</i> , common bean, Maize	Singh <i>et al.</i> , 2017
	Methionine and cysteine	Sulfur-rich protein, S2SA	Sesame (<i>Sesamum indicum</i>)	Lee <i>et al.</i> , 2003
Wheat	Vitamin A	psy1, crtI, CrtB+ CrtI	Maize, bacteria	Cong <i>et al.</i> , 2009 ^[17]
	Amylose	SBEIIa	Wheat	Sestili <i>et al.</i> , 2010
	Anthocyanin	Dhn12, Itr1 and Ltp1	Barley	Doshi <i>et al.</i> , 2006 ^[22]
Maize	Vitamin E	HGGT	Barley	Cahoon <i>et al.</i> , 2003 ^[12]
	Lysin and total protein	Sb401	<i>Solanum berthaultii</i>	Yu <i>et al.</i> , 2004
	Lipid, protein (lysine) and starch	AtGIF1, OstGIF1, ZmGIF1	<i>Arabidopsis</i> , rice, maize	
Sorghum	Lysin, vitamin A, Fe and Zn	PSY1, CRTI, At-DXS HGGT	Maize, <i>Pantoea ananatis</i> , <i>Arabidopsis</i> , barley	Zhao <i>et al.</i> , 2019
	Asparagine content	ENGase	CRISPR/Cas9	Kapusi <i>et al.</i> , 2017 ^[39]

Limitations in Transgenic approaches:

1. Transgenic crops have the advantage of overcoming restricted genetic variation among plants in conventional breeding. However, a major limitation of transgenic methods is the low acceptance among the masses. The adoption of bio-fortified crops by farmers and communities is crucial for improving nutritional health, but low acceptance hinders widespread adoption (Babili *et al.*, 2004).
2. Different countries have varying regulatory processes for the acceptance and commercialization of transgenic crops. The current political and economic landscape is not receptive to transgenic technology (Inaba *et al.*, 2004)^[37].
3. Regulatory processes for transgenic crops are expensive and time-consuming (Watanabe *et al.*, 2005). Bt Brinjal, developed by Mahyco in India, faced opposition and a moratorium on its release due to concerns raised by scientists, farmers and anti-GMO activists. Four varieties of Bt Brinjal received approval for commercial release in Bangladesh, contrasting with the situation in India.
4. The success rate of transgenic-based approaches in terms of cultivar release is low due to the time required for various stages, including target trait and gene identification, modification, expression and assessment of agronomical traits.
5. Golden Rice, a transgenic crop, has faced delays in its release due to issues with its yield and the inability to obtain government approval for dissemination.

Future Perspectives

Going forward, it is imperative to develop robust communication and outreach strategies that integrate ethical considerations pertaining to bio-fortified staple food production and consumption. Diverse countries may necessitate context-specific approaches to make bio-fortified food products acceptable and economically accessible. The public sector must assume a leadership role in formulating evidence-based policies and regulatory frameworks to foster consumer adoption of bio-fortified varieties. Furthermore, forthcoming research should prioritize integration of agronomic and genomic strategies to improve mineral translocation in crops and elucidate the physiological mechanisms governing mineral accumulation in harvestable plant organs. Such efforts will accelerate adoption of bio-

fortification programs and more effectively address micronutrient insufficiencies. Despite existing constraints, bio-fortification harbours immense capacity to deliver nutritionally superior food to billions globally, advancing the goal of a world free from hidden hunger.

Conclusion

Bio-fortification represents an economically viable and ecologically sustainable strategy for enhancing crop nutritional composition and alleviating malnutrition at the global scale. It employs diverse techniques spanning plant genetic improvement, transgenic engineering and targeted mineral fertilizer application. Nevertheless, the implementation of bio-fortification remains a complex undertaking, necessitating interdisciplinary collaboration among agronomists, plant breeders, molecular biotechnologists, genetic engineers and nutrition scientists. Although transgenic approaches are considered superior to classical breeding for crop bio-fortification, they encounter significant hurdles related to regulatory approval and public acceptance. The commercialization of transgenic bio-fortified rice varieties has been restricted, reflecting stringent regulatory impediments. For bio-fortification to achieve meaningful nutritional impact, the developed cultivars must be systematically mainstreamed into formal seed supply chains and assimilated into both formal and smallholder farming systems. Multi-bio-fortification, which concurrently enriches crops with several micronutrients, provides a comprehensive strategy for addressing multi-faceted nutritional deficiencies. Consolidating the bio-fortification program demands focused research into agronomic-genomic integration and a deeper mechanistic understanding of mineral homeostatic regulation in crop plants.

References

1. S, Mangrauthia SK, Sarla N. Expression profiling of iron deficiency responsive micro RNAs and gene targets in rice seedlings of Madhukar × Swarna recombinant inbred lines with contrasting levels of iron in seeds. *Plant Soil*. 2015;396:1–14.
2. Al Noor M, Quadir QF, Naher J, Jewel ZA, Rashid HO, Chakrobarty T, *et al.* Inducing variability in rice for

- enriched iron and zinc content through *in vitro* culture. Plant Tissue Cult Biotechnol. 2019;29:161–174.
3. Al-Babili S, Beyer P. Golden rice on the road—five years to go? Trends Plant Sci. 2004;10(12):565–573.
 4. Ambati D, Prasad SS, Singh JB, Verma DK, Mishra AN, Prakasha TL, Phuke RM, Sharma KC, Singh AK, *et al.* High-yielding durum wheat variety HI 8759 PusaTejas—A new rust and Karnal bunt resistant. Indian Farming. 2019;69:20–22.
 5. Anderson MS, Saltzman A, Virk PS, Pfeiffer WH. Progress update: Crop development of biofortified staple food crops under HarvestPlus. Afr J Food Agric Nutr Dev. 2017;17:11905–11935.
 6. Aziz MZ, Yaseen M, Abbas T, Naveed M, Mustafa A, Hamid Y, *et al.* Foliar application of micronutrients enhances crop stand, yield and the biofortification essential for human health of different wheat cultivars. J Integr Agric. 2019;18(6):1369–1378.
 7. Bana RS, Jat GS, Grover M, Bamboriya SD, Singh D, Bansal R, *et al.* Foliar nutrient supplementation with micronutrient-embedded fertilizer increases biofortification, soil biological activity and productivity of eggplant. Sci Rep. 2021;12(1):1–16.
 8. Beyer P, Al-Babili S, Ye X, Lucca P, Schaub P, Welsch R, Potrykus I. Golden rice: introducing the β -carotene biosynthesis pathway into rice endosperm by genetic engineering to defeat vitamin A deficiency. J Nutr. 2002;132(3):506S–510S.
 9. Boonyaves K, Gruissem W, Bhullar NK. NOD promoter-controlled AtIRT1 expression functions synergistically with NAS and FERRITIN genes to increase iron in rice grains. Plant Mol Biol. 2016;90:207–215.
 10. Bouis HE, Saltzman A. Improving nutrition through biofortification: a review of evidence from HarvestPlus, 2003 through 2016. Glob Food Sec. 2017;12:49–58.
 11. Bull SE, Seung D, Chanez C, Mehta D, Kuon JE, Truernit E, *et al.* Accelerated ex situ breeding of GBSS- and PTST1-edited cassava for modified starch. Sci Adv. 2018;4(9):eaat6086.
 12. Cahoon EB, Hall SE, Ripp KG, Ganzke TS, Hitz WD, Coughlan SJ. Metabolic redesign of vitamin E biosynthesis in plants for tocotrienol production and increased antioxidant content. Nat Biotechnol. 2003;21:1082–1087.
 13. Cakmak I, Kutman UB. Agronomic biofortification of cereals with zinc: a review. Eur J Soil Sci. 2018;69(1):172–180.
 14. Chattha MU, Hassan MU, Khan I, Chattha MB, Mahmood A, Chattha MU, *et al.* Biofortification of wheat cultivars to combat zinc deficiency. Front Plant Sci. 2017;8:281.
 15. Chen R, Xue G, Chen P, Yao B, Yang W, Ma Q, *et al.* Transgenic maize plants expressing a fungal phytase gene. Transgenic Res. 2008;17:633–643.
 16. Chinnusamy V, Zhu JK. Epigenetic regulation of stress responses in plants. Curr Opin Plant Biol. 2009;12(2):133–139.
 17. Cong L, Wang C, Chen L, Liu H, Yang G, He G. Expression of phytoene synthase1 and carotene desaturase crtI genes result in an increase in the total carotenoids content in transgenic elite wheat (*Triticum aestivum* L.). J Agric Food Chem. 2009;57:8652–8660.
 18. Das AK, Hossain F, Muthusamy V, Zunjare RU, Chauhan HS, Baveja A, *et al.* Genetic analyses of kernel tocopherols in maize possessing novel allele of γ -tocopherol methyl transferase (ZmVTE4). In: International Symposium on Biodiversity and Biobanking—Biodiverse 2018; 2018 Jan 27–29; Guwahati, India. Guwahati: IIT Guwahati; 2018. p. 112–113.
 19. De Valença AW, Bake A, Brouwer ID, Giller KE. Agronomic biofortification of crops to fight hidden hunger in sub-Saharan Africa. Glob Food Sec. 2017;12:8–14.
 20. Deshmukh R, Nadaf A, Ansari WA, Singh K, Sonah H, editors. Biofortification in cereals: progress and prospects. Singapore: Springer Nature Singapore; 2023.
 21. Di GF, Petropoulos SA, Ozores-Hampton M, Morgan K, Roskopf EN. Zinc and iron agronomic biofortification of Brassicaceae microgreens. Agronomy. 2019;9(11):677.
 22. Doshi KM, Eudes F, Laroche A, Gaudet D. Transient embryo-specific expression of anthocyanin in wheat. Vitro Cell Dev Biol Plant. 2006;42:432–438.
 23. Duan G, Wang X, Tang K. MET18 connects the cytosolic iron-sulfur cluster assembly pathway to active DNA demethylation in Arabidopsis. PLoS Genet. 2015;11(10):e1005559.
 24. FAO. World food and agriculture statistical yearbook 2020. Rome: FAO; 2020.
 25. Fischer JJ, Beatty PH, Good AG. Manipulation of microRNA expression to improve nitrogen use efficiency. Plant Sci. 2013;210:70–81.
 26. Frison EA. From uniformity to diversity: a paradigm shift from industrial agriculture to diversified agroecological systems. 2016;29:237.
 27. Gande NK, Kundur PJ, Soman R. Identification of putative candidate gene markers for grain zinc content using recombinant inbred lines population of IRRI38 $\times$ Jeerigesanna. Afr J Biotechnol. 2014;13(5):657–663.
 28. Garg M, Sharma N, Sharma S, Kapoor P, Kumar A, Chunduri V, *et al.* Biofortified crops generated by breeding, agronomy and transgenic approaches are improving lives of millions of people around the world. Front Nutr. 2018;5:12.
 29. Giordano M, El-Nakhel C, Pannico A, Kyriacou MC, Stazi SR, De PS, *et al.* Iron biofortification of red and green pigmented lettuce in closed soilless cultivation impacts crop performance and modulates mineral and bioactive composition. Agronomy. 2019;9(6):290.
 30. Govindaraj M, Rai KN, Shanmugasundaram P. Intra-population genetic variance for grain iron and zinc contents and agronomic traits in pearl millet. Crop J. 2016;4(1):48–54.
 31. Gregorio GB, Senadhira D, Htut H, Graham RD. Breeding for trace mineral density in rice. Food Nutr Bull. 2000;21:382–386.
 32. Grujic D, Yazici AM, Tutus Y, Cakmak I, Singh BR. Biofortification of silage maize with zinc, iron and selenium as affected by nitrogen fertilization. Plants. 2021;10(2):391.
 33. Gupta HS, Raman B, Agrawal PK, Mahajan V, Hossain F, Thirunavukkarasu N. Accelerated development of quality protein maize hybrid through marker-assisted introgression of opaque-2 allele. Plant Breed. 2013;13:277–282.

34. Haun W, Coffman A, Clasen BM, Demorest ZL, Lowy A, Ray E, *et al.* Improved soybean oil quality by targeted mutagenesis of the fatty acid desaturase 2 gene family. *Plant Biotechnol J.* 2014;12(7):934–940.
35. Hefferon KL. Nutritionally enhanced food crops: progress and perspectives. *Int J Mol Sci.* 2015;16(2):3895–3914.
36. Hornick SB. Factors affecting the nutritional quality of crops. *Am J Altern Agric.* 1992;7:63–68.
37. Inaba M, Macer D. Policy, regulation and attitudes towards agricultural biotechnology in Japan. *J Int Biotechnol Laws.* 2004;1(2):45–53.
38. Johnson AAT, Kyriacou B, Callahan DL. Constitutive overexpression of the OsNAS gene family reveals single-gene strategies for effective iron- and zinc-biofortification of rice endosperm. *PLoS One.* 2011;6:e24476.
39. Kapusi E, Corcuera-Gómez M, Melnik S, Stoger E. Heritable genomic fragment deletions and small indels in the putative ENGase gene induced by CRISPR/Cas9 in barley. *Front Plant Sci.* 2017;8:540.
40. Kaur T, Rana KL, Kour D, Sheikh I, Yadav N, Yadav AN. Microbe-mediated biofortification for micronutrients: present status and future challenges. In: *Trends of microbial biotechnology for sustainable agriculture and biomedicine systems: perspectives for human health.* Amsterdam: Elsevier; 2020. p. 1–17.
41. Kehr J. Systemic regulation of mineral homeostasis by microRNAs. *Front Plant Sci.* 2013;4:145.
42. Lawson PG, Daum D, Czauderna R, Meuser H, Härtling JW. Soil versus foliar iodine fertilization as a biofortification strategy for field-grown vegetables. *Front Plant Sci.* 2015;6:450.
43. Lee S, Kim YY, Lee Y, An G. Rice P1B-type heavy-metal ATPase, OsHMA9, is a metal efflux protein. *Plant Physiol.* 2007;145:831–842. doi:10.1104/pp.107.102236.
44. Lee TTT, Wang MMC, Hou RCW, Chen LJ, Su RC, Wang CS, Tzen JTC. Enhanced methionine and cysteine levels in transgenic rice seeds by the accumulation of sesame 2S albumin. *Biosci Biotechnol Biochem.* 2003;67:1699–1705.
45. Li X, Wang Y, Chen S, Tian H, Fu D, Zhu B, *et al.* Lycopene is enriched in tomato fruit by CRISPR/Cas9-mediated multiplex genome editing. *Front Plant Sci.* 2018;9:559. doi:10.3389/fpls.2018.00559.
46. Liu BH. Statistical genomics: linkage mapping and QTL analysis. Boca Raton: CRC Press; 1997.
47. Mao H, Wang J, Wang Z, Zan Y, Lyons G, Zou C. Using agronomic biofortification to boost zinc, selenium and iodine concentrations of food crops grown on the loess plateau in China. *J Soil Sci Plant Nutr.* 2014;14(2):459–470.
48. Mayer JE, Pfeiffer WH, Beyer P. Biofortified crops to alleviate micronutrient malnutrition. *Curr Opin Plant Biol.* 2008;11:166–170.
49. McGuire S. FAO, IFAD and WFP. The state of food insecurity in the world 2015: meeting the 2015 international hunger targets: taking stock of uneven progress. Rome: FAO; 2015. *Adv Nutr.* 2015;6(5):623–624.
50. Okuzaki A, Ogawa T, Koizuka C, Kaneko K, Inaba M, Imamura J, *et al.* CRISPR/Cas9-mediated genome editing of the fatty acid desaturase 2 gene in *Brassica napus*. *Plant Physiol Biochem.* 2018;131:63–69. doi:10.1016/j.plaphy.2018.04.025.
51. Pandey N, Hossain F, Kumar K, Vishwakarma AK, Nepolean T, Vignesh M, *et al.* Microsatellite marker-based genetic diversity among quality protein maize (QPM) inbred lines differing for kernel iron and zinc. *Mol Plant Breed.* 2015;6:1–10.
52. Paul S, Ali N, Gayen D, Datta SK, Datta K. Molecular breeding of *Osfer2* gene to increase iron nutrition in rice grain. *GM Crops Food.* 2012;3(4):310–316.
53. Perez-Massot E, Banakar R, Gómez-Galera S, Zorrilla-López U, Sanahuja G, Arjó G, *et al.* The contribution of transgenic plants to better health through improved nutrition: opportunities and constraints. *Genes Nutr.* 2013;8:29–41. doi:10.1007/s12263-012-0315-5.
54. Pfeiffer WH, McClafferty B. HarvestPlus: breeding crops for better nutrition. *Crop Sci.* 2007;47:S88.
55. Rai KN, Patil HT, Yadav OP, Govindaraj M, Khairwal IS, Cherian B, *et al.* Notification of crop varieties and registration of germplasm: pearl millet variety ‘Dhanashakti’. *Indian J Genet Plant Breed.* 2014;74(3):405–406.
56. Ram S, Mishra B. Cereals processing and nutritional quality. New Delhi: New India Publishing Agency; 2010. p. 188.
57. Ram H, Kaur M, Gupta N, Kumar B. Integrated approaches for biofortification of food crops by improving input use efficiency. In: *Input use efficiency for food and environmental security.* Singapore: Springer; 2021. p. 421–440.
58. Ramesh SA, Choimes S, Schachtman DP. Overexpression of an *Arabidopsis* zinc transporter in *Hordeum vulgare* increases short-term zinc uptake after zinc deprivation and seed zinc content. *Plant Mol Biol.* 2004;54:373–385. doi:10.1023/B:PLAN.0000036370.70912.34.
59. Saleem I, Javid S, Bibi F, Ehsan S, Niaz A, Ahmad ZA. Biofortification of maize grain with zinc and iron by using fertilizing approach. *J Agric Ecol Res Int.* 2016;7(4):1–6.
60. Saltzman A, Birol E, Bouis HE, Boy E, De Moura FF, Islam Y, Pfeiffer WH. Biofortification: progress toward a more nourishing future. *Glob Food Secur.* 2013;2(1):9–17.
61. Sánchez-León S, Gil-Humanes J, Ozuna CV, Giménez MJ, Sousa C, Voytas DF, *et al.* Low-gluten, nontransgenic wheat engineered with CRISPR/Cas9. *Plant Biotechnol J.* 2018;16(4):902–910. doi:10.1111/pbi.12837.
62. Sestili F, Janni M, Doherty A, Botticella E, D’Ovidio R, Masci S, Lafiandra D. Increasing the amylose

- content of durum wheat through silencing of the *SBEIIa* genes. *BMC Plant Biol.* 2010;10:144.
63. Shukla AK, Behera SK, Prakash C, Tripathi A, Patra AK, Dwivedi BS, *et al.* Deficiency of phyto-available sulphur, zinc, boron, iron, copper and manganese in soils of India. *Sci Rep.* 2021;11(1):1-13.
 64. Singh SK, Uddeen R. Nutri-farms for mitigating malnutrition in India. In: *Biofortification of food crops.* New Delhi: Springer; 2016. p. 461-477.
 65. Singh SP, Gruissem W, Bhullar NK. Single genetic locus improvement of iron, zinc and β -carotene content in rice grains. *Sci Rep.* 2017;7:147.
 66. Sun Y, Jiao G, Liu Z, Zhang X, Li J, Guo X, *et al.* Generation of high-amylose rice through CRISPR/Cas9-mediated targeted mutagenesis of starch branching enzymes. *Front Plant Sci.* 2017;8:298. doi:10.3389/fpls.2017.00298.
 67. Tanaka H, Yabuta Y, Tamoi M, Tanabe N, Shigeoka S. Generation of transgenic tobacco plants with enhanced tocotrienol levels through the ectopic expression of rice homogentisate geranylgeranyl transferase. *Plant Biotechnol.* 2015;32:233-238. doi:10.5511/plantbiotechnology.15.0702a.
 68. Tariq M, Hameed S, Malik KA, Hafeez FY. Plant root associated bacteria for zinc mobilization in rice. *Pak J Bot.* 2007;39(1):245.
 69. Veillet F, Chauvin L, Kermarrec MP, Sevestre F, Merrer M, Terret Z, *et al.* The *Solanum tuberosum* *GBSSI* gene: a target for assessing gene and base editing in tetraploid potato. *Plant Cell Rep.* 2019;38(9):1065-1080. doi:10.1007/s00299-019-02426-w.
 70. Wang Y, Mei S, Wang Z. Metabolite profiling of a zinc-accumulating rice mutant. *J Agric Food Chem.* 2017;65:3775. doi:10.1021/acs.jafc.7b00105.
 71. Wang D, Samsulrizal N, Yan C, Allcock NS, Craigon J, Blanco-Ulate B, *et al.* Characterisation of CRISPR mutants targeting genes modulating pectin degradation in ripening tomato. *Plant Physiol.* 2019;179(2):544-557. doi:10.1104/pp.18.01187.
 72. Watanabe KN, Sassa Y, Suda E, Chen CH, Inaba M, Kikuchi A. Global political, economic, social and technological issues on transgenic crops—review. *Plant Biotechnol.* 2005;22(5):515-522. doi:10.5511/plantbiotechnology.22.515.
 73. Wirth J, Poletti S, Aeschlimann B. Rice endosperm iron biofortification by targeted and synergistic action of nicotianamine synthase and ferritin. *Plant Biotechnol J.* 2009;7:631-644.
 74. Wirth J, Poletti S, Aeschlimann B, Yakandawala N, Drosse B, Osorio S, *et al.* Rice endosperm iron biofortification by targeted and synergistic action of nicotianamine synthase and ferritin. *Plant Biotechnol J.* 2009;7:631-644. doi:10.1111/j.1467-7652.2009.00430.x.
 75. Yadava DK, Choudhury PR, Hossain F, Kumar D. *Biofortified varieties: sustainable way to alleviate malnutrition.* New Delhi: Indian Council of Agricultural Research; 2017.
 76. Yu J, Peng P, Zhang X, Zhao Q, Zhu D, Sun X, Liu J, Ao G. Seed-specific expression of a lysine-rich protein *sb401* gene significantly increases both lysine and total protein content in maize seeds. *Mol Breed.* 2004;14:1-7.
 77. Yuan M, Zhu J, Gong L, He L, Lee C, Han S, *et al.* Mutagenesis of *FAD2* genes in peanut with CRISPR/Cas9-based gene editing. *BMC Biotechnol.* 2019;19(1):24. doi:10.1186/s12896-019-0516-8.
 78. Zhao ZY, Che P, Glassman K, Albertsen M. Nutritionally enhanced sorghum for the arid and semiarid tropical areas of Africa. In: *Sorghum.* New York, NY: Humana Press; 2019. p. 197-207.
 79. Zunjare RU, Hossain F, Muthusamy V, Baveja A, Chauhan HS, Bhat JS, *et al.* Development of biofortified maize hybrids through marker-assisted stacking of β -carotene hydroxylase, lycopene- ϵ -cyclase and *Opaque2* genes. *Front Plant Sci.* 2018;9:178.
 80. Zuo Y, Liu Y, Zhang F, Christie P. A study on the improvement of iron nutrition of peanut intercropping with maize on nitrogen fixation at early stages of growth of peanut on calcareous soil. *Soil Sci Plant Nutr.* 2004;50(7):1071-1078.