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Cereal Nutrient Enhancement through biofortification: Enhancing public health via improved nutrient content and quality- A comprehensive review

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ABSTRACT

Hidden hunger, or micronutrient deficiency, remains a pervasive and insidious challenge afflicting over half of the global population. It is a formidable barrier to the well-being of communities worldwide, threatening their health and prosperity. In this comprehensive review, we delve into the realm of biofortification, a sustainable solution with the potential to ameliorate this pressing issue and enhance the overall quality of food. Biofortification is the practice of enhancing the nutrient content of crops by integrating vital vitamins and minerals into their genetic makeup. The focus of our analysis primarily revolves around nutrients such as vitamin A (retinol), iron (Fe), lysine, tryptophan, zinc (Zn) and others that are essential for daily human functioning. The review meticulously examines the current scientific literature, revealing the far-reaching implications of biofortification in combatting malnutrition and hidden hunger. Furthermore, this study underscores the importance of integrating biofortified crop varieties into agricultural systems. A multitude of recommendations and future prospects, delineated by various authors, are meticulously organized and categorized, serving as a foundation for new research directions and strategies aimed at enhancing crop biofortification. These strategies are of paramount importance, as they strive to address the monumental challenge of combining nutrient density with high crop yields and profitability, a task that has proven to be exceptionally intricate for plant breeders. A key emphasis is placed on encouraging policymakers and stakeholders to consider biofortification as a pivotal component in their efforts to reduce micronutrient deficiencies. Investments in developing countries, aimed at promoting the adoption and consumption of biofortified crop varieties, are vital steps toward achieving this objective. According to the Consultative Group of International Agricultural Research, there is an urgent need to enhance the genetic potential of staple crops, fortify their nutrient content, and foster their widespread adoption.

KEY WORDS

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Introduction

The term "biofortification" or "biological fortification" pertains to the enhancement of nutrients in food crops, increasing their bioavailability to humans. This is achieved through the utilization of contemporary biotechnology methods, conventional plant breeding, and agricultural practices. As per the United Nations Food and Agriculture Organization, roughly 792.5 million individuals globally grapple with malnutrition, with 780 million residing in developing nations [1]. Additionally, about two billion people worldwide experience "hidden hunger," which arises from an insufficient intake of essential micronutrients in their daily diets, despite the increased production of food crops [2-4]. Furthermore, the issue of overnutrition is progressively gaining prominence.

Biofortification for Addressing the Global Issue of Hidden Hunger

Historically, the distribution of vitamins and minerals to the general population has primarily occurred through nutrient supplementation initiatives. However, these programs do not align with the objectives of international health organizations, as they depend on external funding that lacks year-to-year certainty. Additional challenges encompass the limited purchasing capacity of underprivileged individuals, barriers in accessing markets and healthcare systems , as well as a deficiency in awareness regarding the enduring health advantages associated with these nutrient supplements [4,5]. Consequently, the process of bio-fortifying various crop varieties offers a sustainable and enduring resolution for delivering micronutrient-rich crops to the populace. Moreover, biofortified crops, boasting elevated bioavailable levels of vital micronutrients, are disseminated to consumers through conventional agricultural and food trade methods. This approach offers a practical means of reaching undernourished and economically disadvantaged households with limited access to diverse diets, supplements, and fortified foods.

From an economic perspective, biofortification represents a singular investment that offers a cost-efficient, enduring, and sustainable strategy to address hidden hunger. This is because there are no ongoing expenses associated with the acquisition of fortifiers or their addition to the food supply during processing once biofortified crops are established [6-12]. Additionally, given the anticipated significant population growth in the developing world in the coming decades and the changing climate conditions, attaining food security will present an even more significant challenge [13,14]. Consequently, organizations such as

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the World Health Organization and the Consultative Group on International Agricultural Research (CGIAR) have prioritized the development of nutritionally enhanced, high-yielding biofortified crops as a central objective [15].

The primary objective of biofortification is to generate ample quantities of wholesome and safe foods [16]. The enrichment of vital micronutrients in crop plants can be achieved through three distinct methods: transgenic, conventional breeding, and agronomic techniques, all of which entail the utilization of biotechnology, traditional crop breeding, and fertilization strategies. Notable crops such as rice, wheat, maize, sorghum, lupine, common bean, potato, sweet potato, and tomato are frequently the focal points of transgenic, conventional breeding, and agronomic methodologies (as depicted in Figure 1).

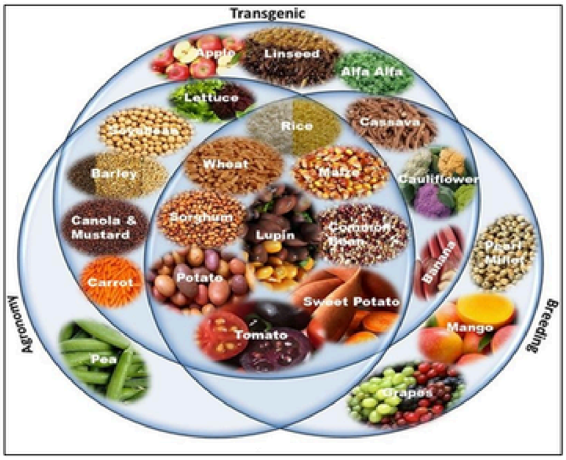

Figure 1. Biofortified crops generated by different approaches for most common vegetables, beans, and fruits have been targeted by all three approaches [17].

Biofortified Cereals

Biofortification in rice

Rice has been specifically chosen to combat the global issue of malnutrition, particularly vitamin deficiency, which is a significant challenge for disadvantaged populations due to limited access to diverse diets. A notable breakthrough in this regard is "golden rice," which serves as an effective source of provitamin A (beta-carotene) and has the potential to reduce the burden of disease by expressing PSY and carotene desaturase genes [18-22]. By targeting the gene responsible for carotene desaturation, the precursor of beta-carotene, phytoene, has been boosted by up to 23-fold [23].

Additionally, folic acid (vitamin B9) is crucial for a healthy pregnancy and the prevention of anemia. Rice has been genetically modified to enhance its folate content (up to 150-fold) through the overexpression of Arabidopsis GTP-cyclohydrolase I (GTPCHI) and aminodeoxychorismate synthase (ADCS) [24,25]. It was found that 100 grams of this modified rice can meet the daily folate requirements of an adult.

Rice has emerged as a potential solution to the global issue of iron deficiency anemia. Numerous studies have shown that the expression of various genes, including those encoding nicotianamine aminotransferase, iron transporter OsIRT1, nicotianamine synthase 1 (OsNAS1) and 2 (OsNAS2), soybean ferritin, and common bean ferritin, can lead to an increase in iron content in rice [17,26-32]. Additionally, iron biofortified rice has been developed by introducing multiple iron nutrition genes [33-35]. Notably, aside from boosting iron content, the presence of antinutrient compounds in rice, such as phytic acid, has been reduced, enhancing the bioavailability of iron [36]. Similarly, by overexpressing OsIRT1 and incorporating mugineic acid synthesis genes from barley (HvNAS1, HvNAS1, HvNAAT-A, HvNAAT-B, IDS3), the zinc content in genetically modified (GM) rice was elevated [37,38].

The enhancement of essential amino acid content in rice has been addressed through the expression of seed-specific genes from various sources, including bean-phaseolin, pea legumin, sesame 2S albumin, soybean glycinin, bacterial aspartate kinase, dihydrodipicolinate synthase (DHPS), maize DHPS, rice anthranilate synthase-subunit, and E. coli [39-46]. Furthermore, rice has also been a focus for improving seed oil quality by augmenting the levels of polyunsaturated fatty acids, which can contribute to reducing bad cholesterol levels and enhancing human nutrition. This objective has been achieved by introducing the soybean omega-3 fatty acid desaturase (FAD3) gene [GmFAD3], increasing the essential fatty acid linolenic acid in rice [47].

Flavonoids, known for their antioxidant properties, have seen an increase in their presence in rice by the expression of maize C1 and R-S regulatory genes, which encompass Myb-type and basic helix-loop-helix-type transcription factors [48]. Additionally, phenylalanine ammonia lyase and chalcone synthase (CHS) genes have been utilized to enhance flavonoid content [49]. To combat the challenges of overnutrition and obesity, rice has been modified to contain less digestible and resistant amylose starch by expressing antisense waxy genes and employing antisense RNA inhibition of starch-branching enzymes (SBE) [50-52]. Beyond micronutrient enhancements, the introduction of functional human milk protein, lactoferrin, in rice grains has paved the way for the development of value-added cereal-based ingredients for use in infant formula and baby food [53].

Biofortification in wheat

Wheat stands as one of the most extensively cultivated staple food crops worldwide. Researchers have turned to wheat to confront nutritional deficiencies, including those related to vitamin A, iron, and high-quality proteins. To bolster wheat's provitamin A content, bacterial PSY and carotene desaturase genes (CrtB, CrtI) have been introduced [54,55]. The iron content of wheat has been elevated through the expression of ferritin genes sourced from soybean and wheat itself [TaFer1-A] [56,57]. Additionally, the upregulation of the phytochrome gene (phyA) has been pursued to enhance iron bioavailability, and phytic acid content has been diminished by inhibiting the wheat ABCC13 transporter [58,59].

In the realm of wheat grain protein content, with a particular focus on essential amino acids like lysine, methionine, cysteine, and tyrosine, enhancements have been achieved by incorporating the Amaranthus albumin gene (ama1) [60]. Additionally, wheat has been a subject of interest in elevating antioxidant activity, achieved through the expression of maize regulatory genes linked to anthocyanin production (C1, B-peru) [61]. Efforts to counter overnutrition and obesity have led to the augmentation of less digestible and resistant amylose starch content in wheat. This has been accomplished through the silencing of the SBE gene (SBEIIa) [62].

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Biofortification in maize

Maize holds a significant position as a staple crop in developing countries, and genetic engineering has played a pivotal role in enhancing its content of vitamins, minerals, quality proteins, and mitigating antinutrient components. To enrich maize endosperm with provitamin A (carotenoids), researchers have employed the expression of bacterial crtB alongside multiple carotenogenic genes [5,63,64]. Additionally, vitamin E and its analogs, potent antioxidants with critical implications for human health, have become a focus of research organizations working on the biofortification of these components in maize crops. The overexpression of homogentisic acid geranylgeranyl transferase (HGGT) has led to an increase in the content of tocotrienols and tocopherols in maize [65].

Vitamin C (l-ascorbic acid), a water-soluble antioxidant, plays a crucial role in cardiovascular function, immune cell development, and iron utilization [66]. Its concentration in corn has been amplified nearly 100-fold by converting oxidized ascorbic acid into its reduced form through the expression of dehydroascorbate reductase (DHAR) [67]. Naqvi et al. achieved the creation of multivitamin corn by engineering three distinct metabolic pathways, resulting in a product with 169-fold the typical amount of beta-carotene, double the typical amount of folate, and 6-fold the typical amount of ascorbate [68].

Antinutrient components can diminish the bioavailability of micronutrients. Researchers have boosted iron bioavailability by expressing soybean ferritin and Aspergillus phytase soybean ferritin alone, and Aspergillus niger phyA2 [69-71]. They've also lowered the expression of ATP-binding cassette transporter and multidrug resistance-associated protein [72]. For instance, the Origin Agritech BVLA4 30101 variety in China has undergone biofortification to reduce phytate levels. Maize's zeins, the most prevalent seed storage proteins, have suboptimal nutritional quality due to their low levels of essential amino acids like lysine and tryptophan. However, the essential amino acid content of maize has increased significantly. This was achieved by introducing sb401 from potato, a single bifunctional expression/silencing transgene cassette, which led to heightened lysine content in maize [73-75]. Additionally, antisense dsRNA targeting alpha-zeins, both 19- and 22-kDa variants, has raised the levels of lysine and tryptophan in maize [76].

The significance of lysine content in maize is underscored by maize varieties rich in lysine, such as MavreaTM YieldGard Maize introduced by Monsanto in Japan and Mexico, as well as MaveraTM Maize (LY038) launched by Renessen LLC (Netherlands) in Australia, Columbia, Canada, Japan, Mexico, New Zealand, Taiwan, and the United States. Methionine, a common protein building block with roles in various cellular processes, has also been augmented in maize by modifying the cis- acting site for Dzs10 [77]. The overall amino acid balance in maize has been enhanced through the expression of milk protein alpha-lactalbumin [39].

Table 1. Crops undergoing biofortification processes.

Crop	Variety	Target Nutrient	Nutrient Range Ppm	Year of Release
Rice	DRR Dhan 45	Zinc	12-16	2016
Wheat	WB 02	Zinc	32.0	2017
		Iron	28.0-32.0	
	HPBW 01	Zinc	32.0	2017
		Iron	28.0-32.0	
Maize	Pusa vivek QPM9	Provitamin A	1.0-2.0	2017
		Lysine	1.5-2.0%	
		tryptophan	0.3-0.4%	
	Pusa HM4	lysine	1.5-2.0%	
		tryptophan	0.3-0.4%	
	Pusa HM8	lysine	1.5-2.0%	
		tryptophan	0.3-0.4%	
	Pusa HM9	lysine	0.3-0.4%	
		tryptophan	1.5-2.0%	

Source: ICAR, New Delhi.

Biofortification in barley

As a representative cereal crop, barley has become a target for enhancing its micronutrient content. The overexpression of zinc transporters has led to increased zinc content [78]. Phytase activity in barley seeds has been elevated through the expression of the phytase gene HvPAPhy, thereby enhancing iron and zinc bioavailability. Additionally, the essential amino acid lysine in barley was boosted by expressing the DHPS gene (dapA) [79-81]. Moreover, glucans, dietary fibers associated with reducing the risk of serious human diseases like cardiovascular disease and type II diabetes, have seen increased content in barley through the overexpression of a cellulose synthase-like gene HvCslF [82].

A noteworthy accomplishment using the RNAi approach involved the development of resistant starch (amylose-only) barley by silencing all SBE genes (SBE I, SBE IIa, SBE IIb) [83].

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Additionally, the expression of 6-desaturase (D6D) has led to an increase in the content of health-promoting polyunsaturated fatty acids, specifically alpha-linolenic acid and stearidonic acid (STA) in barley [84]. Efforts have also been made to target the expression of the human lactoferrin gene (HLF) in barley [85]. Furthermore, barley has demonstrated its capability to express numerous bioactive substances with medicinal and industrial significance, such as enzymes and antibiotics.

Biofortification in sorghum

Sorghum plays a vital role as a staple food for millions of rural people, particularly in impoverished regions, due to its ability to thrive in harsh environments. Efforts have been made to enhance its provitamin A (beta-carotene) content through the expression of Homo188-A [86]. Additionally, the inclusion of a high-lysine protein has resulted in an increased content of the essential amino acid lysine in sorghum [HT12] [86]. One challenge associated with sorghum consumption is its comparatively lower digestibility in comparison to other major staple crops, primarily due to the presence of protease-resistant seed storage proteins known as kafirin. To address this issue, the digestibility index of transgenic sorghum has been improved by employing RNAi silencing of kafirin and utilizing combined suppression that involves three genes: kafirin-1, kafirin-2, and kafirin A1 [87,88].

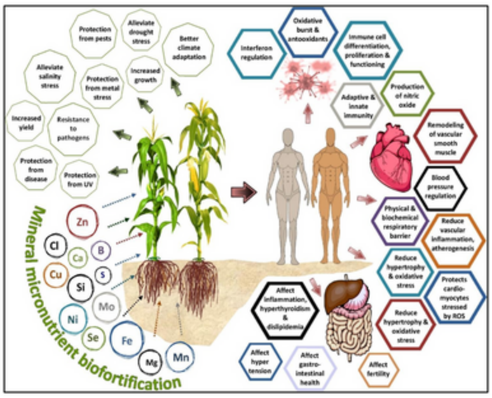

Figure 2. Influence of mineral micronutrient biofortification on the plant physiological processes and its relation to human health and immunity [89].

Sorghum (*Sorghum bicolor L. Moench*) is a vital food crop in arid and semi-arid regions of Asia and Africa and is ranked as the 4th most consumed cereal. Its grain is recognized for its richness in starch, protein, crude fiber, and various micronutrients. However, a significant portion of iron and zinc is lost during the decorticating process, which involves the removal of these nutrients from the aleurone layer and scutellum. Additionally, the bioavailability of iron and zinc from sorghum is relatively low, estimated at around 5% for iron and 20% for zinc. This reduced bioavailability is largely attributed to the inhibitory effect of anti-nutrients such as phytates, which form insoluble complexes with these essential micronutrients.

Table 2. List of genomic approaches in biofortification in cereals (rice, wheat and maize).

Crop	Genome- editing	Nutrients	Gene	Method of transformation	Vectors used
		Carotenoid	-	Particle bombardment	-
		High amylose	SBEIIb		pCXUN-Cas9
		Low phytic acid	OsITPK6	Agrobacterium mediated	pH_itpk6
Rice	Crispr/cas9	Beta- carotene	Osor		-
		Amylose	Waxy		CRISPR/Cas9 vector
		Sucrose efflux transporter	OsSWEET11, OsSWEET14	Agrobacterium transformation	pTOPO/D
		Amylase synthase	OsU3, OsU6a,		pCAMBIA1300
Wheat	Crispr/cas9	Low gluten	Alpha gliadin	Biolistic transformation	pANIC-6E destination vector
		Fe, mg	TaVIT2	Agrobacterium	pBract202
		Carotenoid	Phytoene synthase	mediated	pMD18-T
Maize	Crispr/cas9	Low phytic acid content	Phytic acid synthesis	Agrobacterium transformation	pEasy blunt vector

Source: (Kadam et al.) [90]

Future Perspective

In the face of rising global food prices, exacerbated by factors like COVID-19-induced lockdowns, climate change, variability in weather patterns, and conflicts, access to alternative, nutritious food remains a challenge, particularly in developing countries. Consequently, there is a pressing need to enhance the nutritional value of adapted cereal crops to combat widespread nutritional deficiencies. Genetic "biofortification" undoubtedly continues to offer a sustainable and cost-effective means of addressing global malnutrition issues compared to other food fortification approaches. Given the relatively low rate of commercialization of biotechnology products in many developing countries, conventional plant breeding is likely to play a prominent role in this endeavor. Additionally, the mainstreaming of Marker Assisted Breeding (MAB) in biofortification programs is essential to expedite the crop improvement processes and enhance the nutritional quality of staple crops. To further facilitate this, cost-effective, sensitive, and high-throughput phenotyping tools should be integrated into the breeding process, especially for analyzing complex micronutrients such as zinc and iron. Crops targeted for biofortification should ideally possess traits preferred by farmers to encourage adoption. Alternatively, biofortification can be integrated into the pipeline breeding approach to ensure that all new crop varieties have the key micronutrients. Above all, the involvement of various end users is crucial to highlight the significance of these output traits and to maximize the benefits derived from biofortified crops in the era of nutrition-sensitive agriculture.

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Vitamin and mineral deficiencies, commonly referred to as hidden hunger, have had a detrimental impact on the nutritional well-being of children and women in developing countries due to limited dietary diversity. Over the past few decades, genome-editing technologies have emerged as a transformative force in addressing micronutrient deficiencies, such as iron, zinc, and vitamin A, in the edible parts of cereal crops. These technologies have continuously improved in terms of cost- cost-effectiveness, speed, and precision. Despite the significant progress in genome-editing technologies, there remains a lack of public understanding and acceptance of these methods for crop modification. This leads to lengthy regulatory processes for the approval of cultivating and consuming genetically edited crops. To gain public trust and acceptance, it is crucial to establish clear guidelines that differentiate between genetically modified (GM) organisms and gene-edited cultivars developed using genome-editing technologies, including CRISPR-Cas9. The fundamental difference lies in whether foreign DNA is introduced into the plant. Both methods involve genetic modification, but GM organisms typically acquire genetic material from different species, while CRISPR-edited organisms only alter the original genetic sequence within their genome. Therefore, CRISPR-edited organisms are virtually indistinguishable from natural allelic variants, which are commonly utilized in developing new cultivars through conventional breeding programs. While genome-editing technologies have made significant advancements, they still face political and regulatory challenges to fully harness their power, efficiency, ease of use, and speed.

Conclusions

The utilization of biofortification is a cost-effective agricultural approach that has been widely acknowledged to improve the nutritional status of undernourished populations around the world. This method includes crop breeding, targeted genetic modification, and mineral fertilizer application to produce biofortified food crops with enhanced nutrient content, such as iron, zinc, selenium, and provitamin A, that can address mineral malnutrition in humans. These initiatives, such as the HarvestPlus program and national campaigns, have played a crucial role in achieving these goals by producing crops that have the potential to increase both the quantity and availability of vital mineral elements in human diets, especially in staple cereal crops. Achieving biofortification of crops is a complex undertaking that requires collaboration among various experts such as plant breeders, nutrition scientists, genetic engineers, and molecular biologists. Traditional breeding methods are currently more widely accepted and have been used to improve the nutritional properties of foods. Although transgenic methods are gaining attention, breeding-based approaches have higher success rates since transgenic fortified crop plants face challenges due to acceptance constraints among consumers and time-consuming regulatory approval processes adopted by different countries. Despite these challenges, biofortified crops have a promising future, as they have the potential to eradicate micronutrient malnutrition among billions of impoverished individuals, particularly in developing nations.

Disclosure Statement

No potential conflict of interest was reported by the authors.

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