



Biofortification of Major Crops through Conventional and Modern Biotechnological Approaches to Fight Hidden Hunger: An Overview

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ABSTRACT

Biofortification, the process of enhancing the nutritional content of crops, offers a promising strategy to combat hidden hunger—micronutrient deficiencies affecting over two billion people globally. This review article explores the biofortification of major crops, focusing on both conventional breeding techniques and modern biotechnological approaches. Conventional methods, such as selective breeding and crossbreeding, have been instrumental in increasing the

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levels of essential micronutrients like iron (Fe) and zinc (Zn) in staple crops such as wheat, rice, and maize. For instance, wild relatives of cultivated wheat, including *Triticum dicoccoides* and *Aegilops tauschii*, have been utilized to significantly enhance Fe and Zn content in modern cultivars. Advancements in biotechnological tools, including genetic engineering, marker-assisted selection (MAS), and genome editing (CRISPR/Cas9), have further accelerated the development of biofortified crops. These technologies enable precise modifications to increase the accumulation of micronutrients and improve nutrient bioavailability. For example, transgenic rice varieties enriched with β -carotene (Golden Rice) and enhanced Fe and Zn content through gene editing showcase the potential of biotechnology in addressing micronutrient deficiencies. The review also highlights ongoing efforts and challenges in the field, such as regulatory hurdles, public acceptance, and the need for comprehensive strategies integrating conventional and modern approaches. Furthermore, it discusses the role of international research organizations and collaborations in facilitating the development and dissemination of biofortified crops. In conclusion, combining conventional breeding with cutting-edge biotechnological innovations presents a robust approach to biofortify major crops, offering a sustainable solution to mitigate hidden hunger and improve global food security. Continued research and multi-disciplinary collaborations are essential to fully realize the potential of biofortification in enhancing human nutrition.

Keywords: *Biofortification; molecular breeding; transgenic; micronutrient; essential vitamins and minerals.*

1. INTRODUCTION

“Biofortification involves breeding essential nutrients into food crops, offering a cost-effective, sustainable, and long-term method to enhance micronutrient intake” [1]. “This approach reduces the number of severely malnourished individuals needing complementary interventions and helps maintain better nutritional status” [1]. “It effectively reaches malnourished rural populations with limited access to fortified foods and supplements by incorporating micronutrient-dense traits into crop varieties with desirable agronomic and consumption characteristics, such as high yield” [2]. “Unlike interventions that start in urban centers, biofortified crops can benefit consumers in both rural and urban areas” [2]. “While biofortified foods may not match the high levels of minerals and vitamins found in supplements or fortified foods, they contribute significantly to increasing daily micronutrient intake adequacy across individuals' life cycles” [3]. “With the Earth's population projected to increase from seven billion to over nine billion by 2050, the number of people facing undernourishment and malnutrition is also expected to rise, particularly due to micronutrient deficiencies” [3]. A 2014 report, “The State of Food Insecurity in the World,” published by FAO, IFAD, and WFP, indicated that approximately 800 million people suffered from undernourishment between 2012 and 2014, with many residing in developing countries [4]. “This malnutrition is largely due to the reliance on staple crops like wheat and rice, which lack

essential mineral micronutrients necessary for proper metabolism” [5]. “Approximately three billion people worldwide are affected by elemental dietary deficiencies, such as inadequate essential minerals and micronutrients” [6]. For instance, iron (Fe) and iodine (I) deficiencies impact over 30% and 17% of the global population, respectively, predisposing individuals to various diseases [6].

“Iron-deficiency anemia (IDA), caused by prolonged consumption of iron-deficient diets, affects around two billion people worldwide, particularly in developing countries, where it impacts about 50% of pregnant women and 40% of preschool children” [7]. “To enhance global nutritional status, strategies such as dietary diversification, food fortification, supplementation, and biofortification have been proposed” [5,6,8]. However, “dietary diversification and food fortification/supplementation are often limited by the target population's poor market access and low income” [8]. Consequently, biofortification of crops emerges as an attractive and cost-effective strategy for developing nutrient-rich crops [9]. “Biofortification through breeding offers a more sustainable solution compared to the indiscriminate use of micronutrient-containing fertilizers, which pose environmental risks and face increasing regulatory constraints” [9]. “Cereals and food legumes, which form major components of human diets in developing nations, complement each other nutritionally” [10]. “Assessing the natural variation in minerals and micronutrients within the crop gene pool is

essential for implementing crop-based biofortification” [10]. “Modern omics tools and technologies, such as proteomics, metabolomics, and ionomics, provide comprehensive data that advance our understanding of elemental composition and the genetic networks involved” [11]. This review will discuss recent progress in crop biofortification, particularly in staple crops like cereals and food legumes, and explore the prospects and challenges ahead [11,12]. Through an integrated approach combining conventional breeding and modern biotechnological techniques, biofortification has the potential to significantly mitigate micronutrient deficiencies and improve global food security.

2. IDENTIFYING NUTRIENT-DENSE CROP GENOTYPES THROUGH ANALYSING CROP GENE POOL

“Various published reports indicate that cultivated *Triticum aestivum* L. and *Triticum turgidum* L. ssp. *durum* (Desf.) Husn. species contain lower quantities of grain iron (Fe) and zinc (Zn) compared to wild *Triticum* and *Aegilops* species” [13–15]. Notably, “wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*) serves as a rich reservoir of micronutrients, particularly Fe and Zn [16,17]. Additionally, species such as *Triticum dicoccoides*, *Aegilops tauschii*, *Triticum monococcum*, and *Triticum boeoticum* are significant potential sources of these essential minerals” [18]. Various researchers [13, 18–20] have reported that wild *Triticum* and *Aegilops* species store significantly higher amounts of iron (Fe) and zinc (Zn)—75% and 60% more, respectively—compared to cultivated bread wheat varieties. For instance, wild *Aegilops kotschyi* accession 3790 contains three times more iron in its grains than *Triticum aestivum* cultivars WH291 and WL711 [21]. Furthermore, synthetic hexaploids derived from *Triticum turgidum* ssp. *durum* and *Aegilops tauschii* exhibit 30% higher grain Fe and Zn content [22].

“Efforts are underway at the International Maize and Wheat Improvement Center (CIMMYT) in Mexico to introgress beneficial genes that enhance Fe and Zn content from synthetic varieties derived from crosses such as *Triticum spelta* × *Triticum dicoccon* and *Aegilops tauschii* × *Triticum dicoccon* into high-yielding wheat cultivars” [23]. In a study by researchers [24], 46 rice genotypes, including cultivated and wild accessions, were screened, revealing that wild rice accessions contain a greater amount of iron (Fe) compared to cultivated genotypes. The

genetic variation for Fe content in wild emmer wheat ranges from 15 to 109 mg/kg based on dry weight [17]. Given the significant variability in grain micronutrients, particularly Fe and Zn, among wild species, it is essential to implement focused strategies for the rapid transfer of these micronutrient-accumulating genes and QTLs into high-yielding popular cultivars. Recognizing the importance of landraces for grain Fe content, an examination of 52 accessions, including commercial cultivars and landraces, revealed a wide range of Fe content from 1.32 ppm to 100.45 ppm. These findings underscore the potential of wild species and landraces to enhance the nutritional quality of cultivated wheat through targeted breeding programs.

“The landrace 'Lal Gotal' was found to have the highest amount of Fe at 100.45 ppm [25]. Likewise, an analysis of 126 brown rice genotypes revealed significant variability in Fe content, ranging from 6.2 to 71.6 ppm, with a local accession exhibiting the highest Fe content” [26]. Greater genetic variation in landraces for Fe content has also been supported by other studies [27]. Traditional cultivars, such as 'Kalabath', 'Noothipattu', 'Koomvalazhi', and 'KDML 105', demonstrated higher Fe content compared to improved rice cultivars. Earlier studies showed that traditional Thai brown rice cultivars (CMU122, CMU123, and CMU124) had greater Fe content, ranging from 7 to 22 mg Fe/kg [28]. Up to threefold variation in Fe content (7.5–24 mg Fe/kg) has been reported in cultivated brown rice [29], whereas the range for white rice was between 3 and 11 mg Fe/kg. In another study, 15 Fe-dense and Fe-normal genotypes of unpolished rice were screened using in vitro digestion/Caco-2 cells, revealing Fe content variations from 14 to 39 µg/g [30,31]. Using a similar method, significant variation in kernel Fe content, ranging from 15.5 to 19.1 mg/kg, was observed in maize [32]. Notably, “the genotypes 'ACR90POOL16-DT' and 'ACR86TZESR-W' showed promising results regarding kernel Fe content in maize. Meng et al. (2005) suggested that black rice has higher Fe content compared to other rice varieties such as red rice, sticky rice, and fine rice. Additionally, husk and chaff contain more Fe content. The grain Fe content of white rice varied between 0.05 and 0.2 µg/grain” [28]. The in vitro digestion/Caco-2 model in wheat has helped identify *Aegilops* derivatives with a 1.5-fold increase in bioavailable Fe. A positive correlation between Fe content and protein and phytate contents was also found [33]. Similarly, “a threefold increase in Fe bioavailability was

recorded in 11 Chinese rice genotypes tested using the Caco-2 cell culture model. Furthermore, the impacts of ascorbic acid application on Fe bioavailability were examined, revealing enhanced Fe bioavailability in polished rice due to ascorbic acid" [34].

A study reported up to a threefold difference in Fe content after evaluating a worldwide wheat collection [35]. Similarly, an analysis of the Fe content in a core collection of wheat showed a range of Fe content varying from 26.26 to 68.78 mg/kg, with the 'Andalucia 344' accession carrying the highest Fe content [23]. Another study investigating "Fe accumulation in 20 wheat genotypes in response to Fe treatment reported that selenate enhanced Fe accumulation. Additionally, two Fe-rich genotypes, 'EMB 38' and 'BRS 264', were found to have higher grain Fe content. Significant genetic variation for Fe content was observed across 109 sub-Saharan African inbreds, particularly mid-altitude (15–159 ppm) and lowland (14–134 ppm) inbreds [36]. In a study based on 3-year trial data" [37], variation for kernel Fe concentration in 30 maize genotypes was analyzed, showing a range from 11.28 to 60.11 mg/kg. Two genotypes, 'HP2' and 'BAJIM 06-17', were identified as stable across multiple environments. Recent studies at ICRISAT showed that Fe content varied from 29.8 to 44.2 mg/kg in sorghum [38] and from 30.1 to 75.7 mg/kg in pearl millet. Similarly, [39] reported adequate variability in Fe content, ranging from 18 to 97 ppm, in advanced breeding lines of pearl millet. In wheat, wild emmer, einkorn, and landraces are known as the richest sources of grain zinc (Zn) content. Furthermore, [17] reported that other wheat species such as *T. dicoccoides*, *Ae. tauschii*, *T. monococcum*, and *T. boeoticum* also contain higher Zn content. High variation in Zn content, ranging from 30 to 118 mg/kg, was recorded after screening 825 accessions of *T. dicoccoides* [17]. In hexaploid wheat, the Zn concentration differed (15–35 ppm) in a wheat germplasm collection that comprised elite breeding lines and germplasm collected across the globe [35]. Significant variation was also reported in a wheat core collection, with the Zn content varying between 16.85 and 60.77 mg/kg. Specifically, the Chinese spring bread cultivar (Hong Duan Mang) showed the highest Zn content [23]. Similarly, "genotypic variation for grain Zn was also noticed among 20 Brazilian wheat genotypes, manifesting a twofold difference within the sample studied. Additionally, the addition of selenium led to an increase in the grain Zn content" [40]. "To understand how

genotype and environment interact to determine grain Zn and Fe contents, elite lines of wheat from CIMMYT were tested in the Eastern Gangetic Plains (EGP) of India under various conditions. The findings showed significant G x E effects on grain Fe and Zn concentrations, with more variation observed across locations for Zn" [41].

Multilocation testing of advanced wheat lines developed from *Triticum spelta*, landraces, and synthetic wheat in South Asia and Mexico revealed high heritabilities for Zn and Fe contents across multiple sites. This increased variation opens up new possibilities for selecting Zn- and Fe-dense lines for future use. Additionally, based on phyto siderophore release, [42] identified two Zn-efficient wheat genotypes, Cross and Rushan. In other staple crops, rice germplasm showed grain Zn variation of 13.5–58.4 mg/kg [43], while aromatic rice ranged between 29 and 37 mg/kg, and pearl millet showed a range of 24.5–64.8 mg/kg. Sorghum exhibited a range of 22.2–32.9 mg/kg in terms of Zn content [38]. "The Zn content in advanced breeding lines of pearl millet showed a large variation, ranging from 22 to 69 ppm [39]. Similarly, variable degrees of loss were reported for Fe (24–60%) and Zn (10–58%) in 15 Thai rice genotypes due to genotypic differences in micronutrient loss during the milling process" [44].

In maize, a high degree of variation in Zn content was observed across 109 inbred lines from sub-Saharan Africa, with levels ranging from 12 to 96 ppm for mid-altitude inbreds and 24 to 96 ppm for lowland inbred lines. [37] reported variation in maize kernel Zn concentration (15.14–52.95 mg/kg) and identified a genotype (IML467) with stable kernel Zn concentration across different environments. Regarding Ca content, [45] found variation from 0.25 to 0.73 g/kg (on a dry weight basis) among 132 wheat genotypes, while [46] observed Ca content ranging from 388 to 640 mg/kg in durum wheat. In hexaploid wheat, 'PBW-396' exhibited the highest Ca content (76.67 mg/100 g dry weight) among 10 tested genotypes [47,48] recorded the range of Ca from 0.35 to 0.50 g/kg in triticale grains based on six field trials. Orange maize varieties were found to contain higher provitamin A than yellow varieties [49].

Similarly, yellow endosperm varieties of sorghum carry higher amounts of provitamin A carotenoids [50]. Genetic variability for provitamin A (0.24–

8.80 µg/g) was evident across 1000 tropical maize germplasms at CIMMYT [51], and [52] estimated the range of provitamin A (15–20 µg/g) in improved lines. The study identified a wide variation in kernel β-carotene (0.02–16.50 µg/g) in 105 maize inbreds from India and CIMMYT, attributing the variation primarily to allelic variation in the crtRB1 3'TE gene [53,54] reported tropical maize lines harboring functional markers crtRB1-50TE and 30TE as a rich source of provitamin A. [55] found significant general combining ability (GCA) effects for provitamin A, indicating the presence of additive gene action. Significant G x E effects were observed for grain carotenoid concentration and associated traits in a maize population derived from the cross DExp x CI7, with higher variation under subtropical conditions than temperate conditions [56]. In a study on wheat, the 'RSP-561' genotype was found to have the highest carotenoid content (105.67 µg/100 g) among 10 genotypes tested [57]. "It is important to not only evaluate nutrients but also consider anti-nutritional factors such as phytates. For instance, the phytate content in maize ranged from 1.98 to 2.46 g/kg in a study evaluating 90 S1 families derived from the BS31 population at two locations [58]. Another study found the average phytate content to be 2.91 g/kg in 54 landraces of maize" [59-61].

In wheat, PA content varied from 200 mg/100 g to 400 mg/100 g in refined flour and 600–1000 mg/100 g in whole flour [62]. Two Iranian cultivars, 'Pavarus' and 'Niknejad', were reported to contain low PA, while 'Estar', 'S-78-11', 'S-79-10', and 'Niknejad' had maximum phytase activity. Another study reported PA content ranging from 0.35 to 1.60 mg/100 g across 10 genotypes investigated, identifying the 'HD2687' genotype as exhibiting the lowest PA content [63]. In barley, genetic variation in PA ranging from 3.85 mg/g to 9.85 mg/g across 100 genotypes was observed, with significant variation attributed to effects exerted by both location and time [64].

"Regarding selenium (Se), immense variability in Se content (5–720 µg/kg) was reported in wheat germplasm, with the majority of this variation credited to soil factors. A survey in Malawi involving 88 different sites observed Se concentration in maize grain to range between 0.005 mg/kg and 0.533 mg/kg [65]. In terms of nutrient uptake efficiency, the wheat genotype "Maris Butler" was reported to be highly efficient at extracting manganese (Mn) from Mn-deficient soil" [66]. Additionally, 'Maris Butler' and 'C8MM'

genotypes of wheat were reported to be highly efficient for Mn uptake. Wheat genotypes PBW550, BW9178, and HD2967 were identified as having high utilization efficiency of Mn during the reproductive phase, making them potentially exploitable for the development of Mn-rich wheat cultivars in the future [131]. A comprehensive study examined a worldwide collection of 1763 diverse germplasm accessions in rice for 16 mineral nutrients in flooded and non-flooded conditions, advocating for the possibilities of rice nutritional enhancement using selection and breeding [67]. Similarly, "a study on 72 pearl millet genotypes revealed a greater range of variation for Ca, Fe, copper (Cu), and Zn, while moderate levels of variation were noticed for Mg, P, K, and Mn. The study advocated multi-environmental testing of diverse accessions when measuring variations in mineral and micronutrient contents due to significant G x E interactions, particularly for Fe and Zn densities" [68].

3. ELUCIDATING THE GENETIC ARCHITECTURE OF NUTRIENT ACCUMULATION VIA QTL MAPPING

Detecting the causative loci (genes or QTLs) that determine the mineral/nutrient content is a crucial step in understanding the genetic makeup of nutrient-related traits (Fig. 1). In this section, we review the existing literature on the mapping of QTLs associated with nutrient traits [69] applied an inductively coupled plasma optical emission spectroscopy (ICP-OES) technique to F4 lines derived from the cross (B84 X Os6-2) in maize and detected significant QTLs controlling the concentrations of phosphorus (P), Fe, Zn, and Mg. The QTL analysis revealed a total of 32 QTLs associated with seven different traits, and the phenotypic variances (PVs) of these QTLs ranged between 6.7–19.9%. Furthermore, the co-localization of some of these QTLs on chromosome 3 offers a promising marker-delimited chromosomal region for immediate utilization in nutritional breeding.

Similarly, "QTLs were discovered from F2:3 lines resulting from the crosses Mu6 X SDM and Mo17 X SDM, which determined Zn and Fe concentration in maize kernels and cobs" [70]. "A joint QTL analysis was also performed using data from the two populations, providing a set of 12 QTLs, the majority of which were observed in both joint- and single-environment analyses" [71] identified three QTLs for grain Fe concentration in a maize recombinant inbred line (RIL)

population B73 X Mo17, also known as IBM. Additionally, they found ten QTLs that control Fe bioavailability, accounting for over 50% of the total phenotypic variation (PV). In another study, [72] examined a 178 – P53-based F2:3 population in maize using an atomic absorption spectrophotometer (AAS) and detected five QTLs, four for Zn and one for Fe content, which accounted for 17.5% (Zn) and 16.8% (Fe) of the observed PV. Furthermore, an integrated analysis of QTL data from five studies revealed 10 meta-QTLs (M-QTLs) governing up to 28% of the observed PV. The investigation emphasized the importance of bins 2.07 and 2.08 in deciphering the genetic architecture of mineral concentration in maize [73,74] performed ionome profiling of over 300 intermated RILs belonging to the IBM population, which led to the mapping of major QTLs for cadmium (Cd), potassium (K), and strontium (Sr) accumulation using a high-resolution genetic map comprising 2161 DNA markers.

4. GENOME-WIDE ASSOCIATION STUDY (GWAS)-BASED DISSECTION OF ELEMENTAL COMPOSITION AND NUTRIENT-RELATED TRAITS

“In association mapping, mapping populations derived from multiple founders, such as NAM and multi-parent advanced generation inter-cross (MAGIC), offer advantages over diversity panels. The latter, due to population structure, is prone to generating spurious associations or false positives” [75]. The MAGIC and NAM populations are increasingly utilized in several crops such as rice, wheat, sorghum, etc. A recent example of a MAGIC population is an eight-parent wheat MAGIC population developed at the National Institute of Agricultural Botany (NIAB) in Cambridge, UK, which comprises a total of 1091 lines [76]. Combining multi-founder populations with genome-wide association studies (GWAS) shows promise in overcoming the challenges typically faced with GWAS. Multi-parent populations have also shown promise in generating genome-wide predictions (GWPs) in crop plants.

5. GENOMIC SELECTION (GS) OR GWPS TO DEVELOP NUTRITIONALLY ENRICHED STAPLE CROPS

Genomic selection (GS), proposed by [77], “is a genome-level improvement strategy that differs from marker-assisted selection (MAS). It does not target specific markers but instead utilizes

high-density genetic variants across the entire genome to exploit genome-wide linkage disequilibrium (LD). GS is a black box approach that aims to provide estimated breeding values (EBVs) of individuals using genome-wide marker data” [78-79]. “In conventional plant breeding, EBVs derived from best linear unbiased predictions (BLUPs) have always been important criteria for selecting worthy individuals. The performance of progenies is considered a better indicator of genetic merit than the individual's performance” [78,80,81]. “In GS, phenotyping, which is usually costly and time-consuming, is only used to train the models, i.e., the training population” [3]. “Genotyping of both training and breeding populations is done using high-density DNA markers” [82]. “Several factors affect the accuracy of genomic EBVs, including genetic composition and size of the training population, types and optimum number of DNA markers to be assayed, appropriate statistical methods used to generate genome-wide predictions (GWPs), and the heritability of the traits. While GS has been extensively implemented in livestock breeding, it is still in the development stage in plants” [79]. Encouraging empirical results are being increasingly made available from a wide range of crops, including maize, barley, wheat, soybean, sugar beet, etc [83–87]. “Regarding provitamin A biofortification, [88] used approximately 200 maize lines for prediction analysis using three statistical approaches: RR-BLUP, LASSO, and EN. They considered three different marker sets for assessing prediction accuracies: genome-wide DNA markers, markers specific to carotenoid biosynthesis in maize, and markers targeting candidate genes associated with carotenoid content in maize grain. The statistical approaches offered similar prediction accuracies, with an average GWP accuracy of 0.43 recorded, and the highest accuracy obtained for β -xanthophylls” [88].

6. RISING “OMICS” TECHNOLOGIES TO REINFORCE PLANT NUTRITIONAL GENOMICS AND BREEDING

In line with the rapid advancements in plant genomics, the recent technological progress in “omics” sciences is also noteworthy [89]. “It is expected that fields such as proteomics, metabolomics, ionomics, and others will significantly enhance crop biofortification. By thoroughly examining “metabolic phenotypes” or “metabotypes” using genome-wide genomics approaches, the aim is to elucidate the underlying genetic mechanisms and intricate

molecular networks” [90,91]. Metabolomics, as described by [92], involves detailed characterization of all the metabolites extracted from the cell. Various strategies, primarily based on mass spectrometry (MS) or nuclear magnetic resonance (NMR), are being employed to quantify plant metabolites [92] have provided a brief review of these metabolite-profiling methods and have proposed metabolomics-assisted breeding as a cost-effective supplement to various breeding strategies, compared to transcriptomics and other emerging "omics" technologies. Recently, over 1000 SMTAs were established through metabolite-based genome-wide association studies (GWAS) across 702 maize lines, and the identified loci were further validated by resequencing, expression analysis (e-QTL), and family-based linkage analysis in two recombinant inbred lines (RILs) [90].

due to their common deficiency in human diets, focus on enhancing uptake efficiency, transport to harvestable tissues, and increasing the bioavailability of minerals within the plant” [93]. “Iron, predominantly present as Fe (III) in soil, needs conversion to Fe (II) for absorption, achieved through two pathways: Strategy I involves Fe (III) reduction followed by Fe (II) absorption, while Strategy II employs phytosiderophores to chelate Fe (III) before absorption, primarily in graminaceous plants. Overexpressing transport and chelating proteins facilitates Fe accumulation” [94]. “Interestingly, crosstalk exists between iron and zinc transport pathways, with increased expression of Fe (III) reductases and iron transporters leading to enhanced zinc accumulation, likely due to elevated nicotianamine synthesis facilitating both metals' mobilization” [94].

7. TRANSGENIC STRATEGIES TOWARDS NUTRITIONAL ENRICHMENT OF CROPS

Transgenic approaches provide the quickest and most precise method for developing high-nutrient crops, complementing mineral fertilization and conventional breeding to improve the problem.

“Another approach involves expressing recombinant proteins to store minerals in bioavailable forms. For example, overexpressing soybean ferritin in rice significantly increased iron levels in grains, even after polishing. However, using a constitutive promoter resulted in elevated iron levels in vegetative tissues rather than grains [95]. Bioavailability is crucial, and the anti-nutritional compound phytic acid reduces mineral bioavailability by chelating minerals in the gut. Hence, strategies involving the expression of ferritin and phytase, an enzyme breaking down phytate, have been developed. Transgenic rice and maize experiments demonstrated doubled iron levels in grains, with increased bioavailability observed in maize kernels” [96,97].

7.1 Enrichment of Minerals

“Enriching plants with organic nutrients primarily involves metabolic engineering, while minerals, sourced from the immediate environment, require a different approach. Transgenic strategies, particularly targeting iron and zinc enrichment

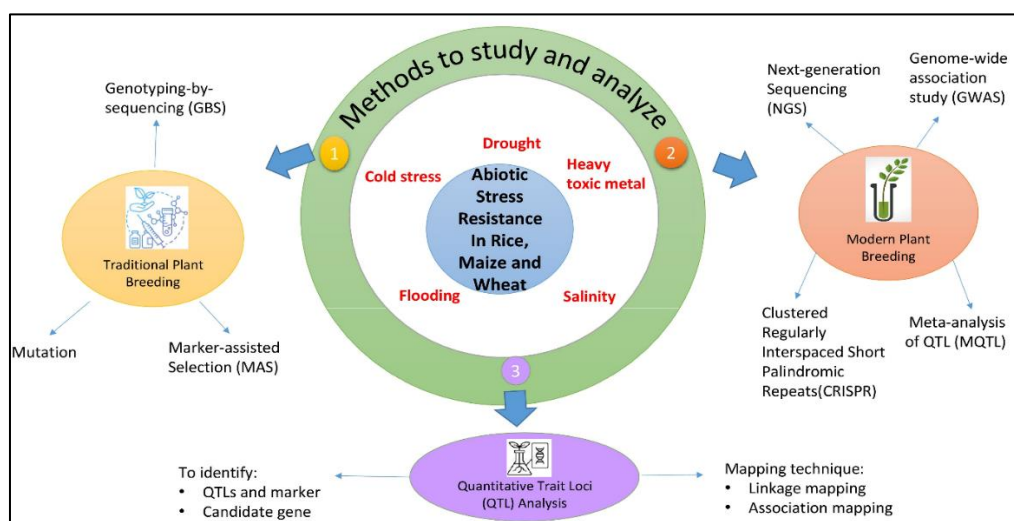


Fig. 1. The article "QTL and candidate genes: Techniques and advancements in breeding major cereals for abiotic stress resistance [135]

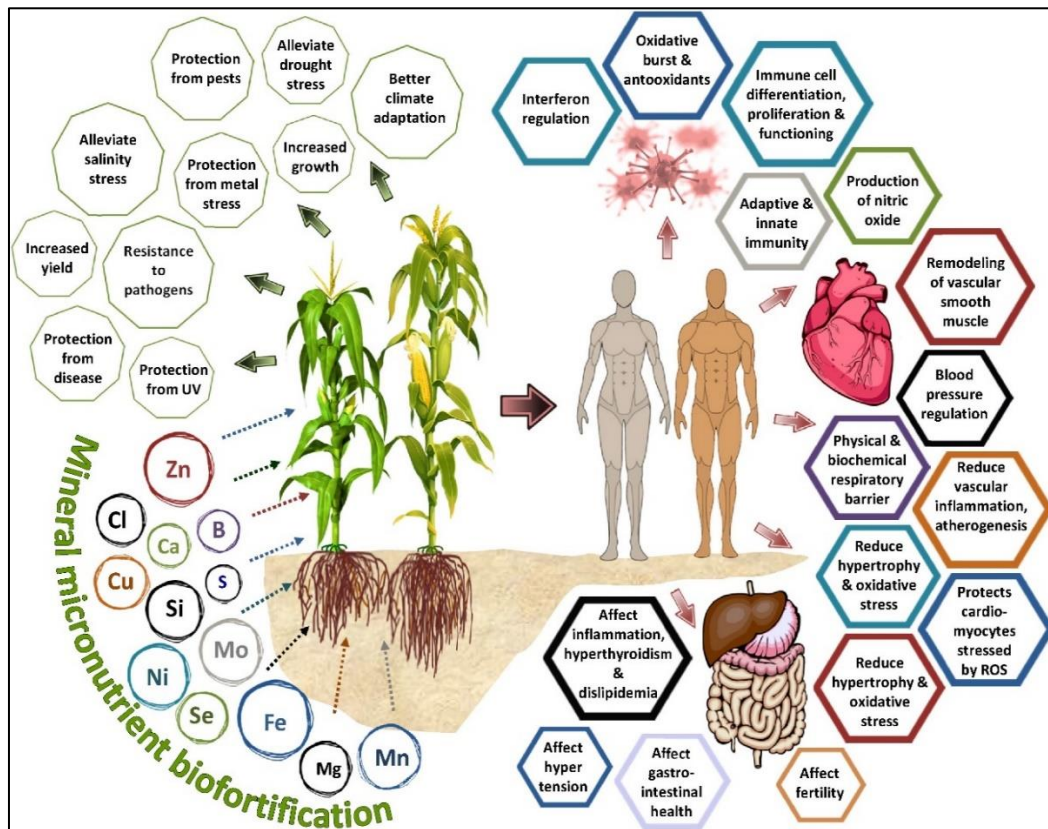


Fig. 2. Influence of micronutrient biofortification to human health and immunity [136]

Similar approaches can be extended to enhance the bioavailability of other minerals, showcasing the potential of transgenic strategies in mineral biofortification [98].

supplementation and fortification, which often face sustainability challenges, biofortification offers a more enduring solution to malnutrition, reaching millions in need in developing nations.

8. GENOMICS-ENABLED BREEDING FOR ENHANCING MICRONUTRIENTS IN CROPS

9. GLOBAL AND NATIONAL STATUS OF MALNUTRITION

“Developing staple foods enriched with essential micronutrients, known as biofortified foods, is a crucial intervention strategy, particularly benefiting vulnerable populations such as resource-poor women, infants, and children” [99,100]. “Recent examinations underscore the genetic potential for augmenting the levels of bioavailable iron, zinc, provitamin A, selenium, and iodine in key staple crops” [101]. This process encompasses various stages, including germplasm screening, studying inheritance patterns, and eventually product development, aimed at introducing new biofortified varieties [101]. “Utilizing existing distribution channels like the public distribution system (PDS) can facilitate the widespread availability of these biofortified foods, minimizing processing expenses” [102]. Unlike conventional approaches like

“More than 840 million people worldwide struggle to access enough food to meet their basic daily energy requirements. Additionally, over three billion individuals face the consequences of micronutrient deficiencies due to limited purchasing power parity (PPP) to afford nutrient-rich foods like meat, fish, fruits, lentils, and vegetables. These economic challenges disproportionately impact women and children across regions such as sub-Saharan Africa, South and Southeast Asia, Latin America, and the Caribbean, leaving them vulnerable to diseases, premature mortality, and cognitive impairments resulting from diets lacking essential nutrients such as iron, vitamin A, iodine, and zinc. In India, which harbors the largest population of malnourished children globally, approximately 1500 children succumb to malnutrition each day. Addressing malnutrition

has the potential to boost India's GDP by an estimated 3%" [103]. India has initiated various governmental interventions, including providing mid-day meals for children, implementing a public distribution system (PDS), and employing agricultural scientific interventions like biofortification.

10. METABOLIC IMPORTANCE OF MAJOR MICRONUTRIENTS IN HUMAN

Macronutrients and micronutrients play vital roles in human growth and metabolic functions. Macronutrients provide chemical energy, while micronutrients serve as essential cofactors or coenzymes necessary for utilizing the energy provided by macronutrients. Micronutrients, named for their requirement in small quantities compared to macronutrients, are integral to overall health.

"Iron serves as a critical component of the catalytic site in most heme and non-heme iron proteins. Iron deficiency affects approximately half of the global population, leading to anaemia according to WHO/UNICEF/UNU 2001. This deficiency can have far-reaching consequences, impacting normal body development, infection resistance, productivity, work capacity, and pregnancy outcomes. Children born to anaemic mothers often lack sufficient iron reserves, requiring more iron than what breast milk provides, leading to growth impairments. Iron-deficiency anaemia (IDA) is prevalent in over 50% of women and preschool children in developing nations, contributing to 20% of maternal deaths during childbirth and hindering physical and mental development in childhood and adolescence" [104,105]. An estimated 800,000 deaths annually are attributed to iron-deficiency anaemia, with endemic infectious diseases further exacerbated in developing regions.

"Zinc serves as a vital functional component in numerous proteins and enzymes involved in DNA replication, gene expression, cellular growth, and differentiation processes. Crucial during periods of rapid growth, inadequate intake during childhood and adolescence can negatively impact growth, sexual development, and psychomotor development. Zinc deficiency is prevalent among children and pregnant women, with severe deficiency leading to stunted growth in children and infants, compromised immunity, impaired vitamin A utilization, dysfunctional vitamin D activity, and increased susceptibility to certain parasitic diseases [106,107]. The body of

evidence regarding zinc deficiency has prompted joint recommendations by WHO and FAO for zinc fortification" [108].

11. APPROACHES TO DEVELOP BIOFORTIFIED CROPS

With recent advancements in tools and technology, biofortification will utilize a wide range of technologies, including agronomic intervention, conventional breeding, molecular marker-assisted breeding, and genetic transformation. The application of this technology varies depending on the crop.

11.1 Genetic Biofortification

Micronutrient malnutrition can be addressed through methods such as food fortification, supplementation, and biofortification. However, conventional approaches often face challenges in reaching economically disadvantaged populations due to funding constraints and distribution issues [109]. Biofortification, considered the most sustainable solution, involves leveraging plant breeding, advanced molecular techniques, and genetic modification to enhance the micronutrient content of food crops [109]. "Genetic biofortification aims to produce crops with increased levels of essential micronutrients, reduced levels of antinutrient compounds, and enhanced nutrient absorption promoters [109]. Success in genetic biofortification relies on the availability of genetic diversity for target traits within the gene pool" [107]. "There is ample potential to enhance the micronutrient density of staple food crops like maize, rice, and wheat through breeding, given the existence of genetic variation for traits such as β -carotene, iron, and zinc" [107]. "Micronutrient traits are generally heritable and stable across environments, making it feasible to combine them with yield traits" [5]. However, "the concentration of micronutrients in edible parts of crops may be influenced by the presence of antinutrient compounds, impacting bioavailability" [107].

11.2 Molecular Marker-Assisted Breeding

Molecular breeding utilizes modern genomic tools and resources, including molecular markers, to enhance the efficiency of conventional breeding [110,111]. DNA markers facilitate precise genotype selection based on linked or flanked markers associated with traits of interest, even at early growth stages [111]. They are also valuable for genetic purity assessment

and diversity analysis [111]. DNA markers, unaffected by environmental factors, have been utilized since the late 1980s to map quantitative traits controlled by multiple genes [112]. "Advanced marker systems like SNP genotyping, DArT marker analysis, and genotyping by sequencing have enabled the identification of Quantitative Trait Loci (QTL) for important agronomic traits in various crops" [112]. These technologies are instrumental in mapping genomic regions/QTLs associated with high micronutrient accumulation and controlling enhancer and antinutrient substances affecting bioavailability [112]. Once identified, marker-assisted selection (MAS) can be employed to transfer targeted QTLs/genes into new crop varieties, thereby facilitating the development of nutrient-rich crops [113].

12. NEW PROSPECTS FOR BIOFORTIFICATION APPROACHES

12.1 Nutri-Genomics

The completion of large-scale genome sequencing projects across various organisms, including plants, bacteria, fungi, and animals, has paved the way for in-depth bioinformatics analysis of genetic sequences. This has given rise to the emerging field of nutrigenomics, which focuses on understanding complex metabolic pathways involved in nutrient production and accumulation in plant tissues as part of biofortification efforts [114]. "Nutrigenomics aims to elucidate the underlying mechanisms governing the synthesis and storage of essential vitamins and minerals within plants. Given the metabolic similarities shared among different organisms through evolution, insights gained from nutrigenomics research in one organism can be extrapolated to others. This comparative understanding serves as a valuable knowledge base for enhancing specific micronutrient levels in crops, particularly benefiting populations in the developing world. Once target genes are identified through nutrigenomics, they can be transferred into crop species to validate their functionality and bring about desired changes in nutritional profiles. This gene transfer process opens avenues for introducing novel traits into breeding programs that may not be present in existing germplasm, thereby broadening the scope of crop improvement efforts" [114].

12.2 Utilization of Next-Generation

NGS technologies have revolutionized breeding pipelines, significantly enhancing the speed and

accuracy of trait mapping and transfer. Over the past five years, both second-generation technologies (SGT) such as Roche/454 FLX Pyrosequencer, GS FLX Titanium/GS Junior, Genome Analyzer (Solexa/Illumina), and Solid Sequencer (Applied Biosystems), as well as third-generation sequencing (TGS) technologies like Ion Torrent PGM/Proton (Life Technologies), HiSeq/MiSeq from Illumina, and Oxford Nanopore Technology, have gained prominence due to their high throughput, extended read lengths, and cost-effectiveness. NGS platforms are now widely employed for diverse applications including de novo sequencing, whole-genome sequencing (WGS), whole-genome resequencing (WGRS), quantitative trait mapping, genome-wide association studies (GWAS), TILLING studies, mutational mapping (MutMap), genotyping by sequencing (GBS), genomic selection (GS), whole-genome bisulfite sequencing (WGBS), reverse and forward genetics analyses, epiQTL analysis, transcriptomics, differential gene expression and epigenetic analysis, small RNA profiling, restriction-site-associated DNA sequencing (RAD-seq), SHORE map, exome sequencing, QTL-seq technology, and marker-trait association studies. Rapid SNP/haplotype and QTL identification approaches have been successfully demonstrated in various crop species through whole-genome resequencing of DNA from diverse mapping panels, leading to the identification of marker-trait associations for agriculturally important traits.

"Micronutrient deficiencies afflict more than two billion individuals worldwide, particularly in regions like sub-Saharan Africa and South Asia, leading to significant health ramifications including low birth weight, anemia, learning impairments, increased morbidity and mortality rates, reduced productivity, and substantial healthcare expenditures. Addressing this "hidden hunger" through crop breeding interventions presents a promising solution. In rural areas of Southeast Asia, deficiencies in essential micronutrients such as iron (Fe), zinc (Zn), selenium (Se), and iodine (I) are prevalent, with approximately 60% of the global population being Fe deficient and 33% Zn deficient. Fe and Zn deficiencies disproportionately affect preschool children and pregnant women due to their heavy reliance on cereal-based diets, which are typically lacking in these vital micronutrients. Combatting these deficiencies necessitates the provision of diets enriched with essential minerals and vitamins. Developing staple food

cultivars fortified with these micronutrients represents a viable strategy to tackle global micronutrient malnutrition through classical plant genetic improvement” [115-119].

13. CROP BIOFORTIFICATION USING BREEDING TECHNIQUES

13.1 Genetic Biofortification Strategies in Lentils

Genetic biofortification employs plant breeding techniques to develop staple food crops with heightened micronutrient levels, reduced antinutrient levels, increased nutrient absorption promoters, and enhanced yield [107]. “Initially, plant breeders assess existing accessions in global germplasm banks to ascertain the presence of sufficient genetic variation for a targeted trait. They then selectively breed nutrient-rich cultivars of major staples, focusing on elevating concentrations of zinc (Zn) and iron (Fe) while enhancing bioavailability. Genetic biofortification, recognized as a long-term and sustainable solution for bolstering mineral bioavailability, particularly in lentils, is widely acknowledged as a cost-effective approach to mitigating mineral deficiencies” [107].

13.2 Harnessing Wild Species for Pre-Breeding in Lentils

Wild relatives harbor valuable alien genes absent in cultivated crops, prompting efforts to collect and preserve them in gene banks [120-130,132,133,134]. “The International Center for Agricultural Research in the Dry Areas (ICARDA) has amassed 587 accessions from six wild *Lens* species across 26 countries. While attempts have been made to identify stress resistance and other traits in cultivated and wild species, the successful integration of alien genes remains limited. To broaden cultivated germplasm diversity, the introgression of alien genes from wild species is essential to mitigate stress epidemics and enhance lentil yields. Advancements in tissue culture techniques have facilitated the introgression of alien genes, warranting urgent pre-breeding initiatives, particularly focusing on wild species harboring valuable alien genes to bolster yield, quality, and stress resilience” [53].

13.3 Utilizing Markers for Biofortification in Lentils

“Marker-assisted selection (MAS) emerges as a potent tool for crop biofortification, offering an

alternative to genetic engineering approaches” [127]. For instance, “genetic markers associated with elevated provitamin A levels in maize have been identified, enabling the selection of maize varieties with increased provitamin A content to address vitamin A deficiencies. While significant strides have been made through conventional breeding, transgenic approaches are deemed necessary and potentially advantageous in certain cases” [128]. Golden Rice, a notable example, showcases the potential of transgenic approaches to address nutritional deficiencies. Ongoing transgenic research in rice explores endosperm-specific promoters to deposit iron, a necessary step to prevent iron loss during milling. Despite these advancements in other crops, lentils have yet to witness significant efforts in marker-assisted biofortification initiatives.

14. CONCLUSION

In conclusion, the endeavor to combat global malnutrition through biofortification strategies represents a promising avenue for addressing hidden hunger, particularly in vulnerable populations across the developing world. Genetic biofortification, facilitated by advancements in plant breeding techniques, offers a sustainable and cost-effective solution to enhance the nutrient content of staple food crops. By harnessing the genetic diversity present in wild relatives and leveraging modern molecular tools like marker-assisted selection, breeders can develop cultivars enriched with essential micronutrients such as iron and zinc, thus fortifying diets and improving public health outcomes. Pre-breeding efforts involving wild species present an opportunity to broaden the genetic base of cultivated crops, enabling the introgression of valuable alien genes to enhance stress resilience and yield potential in lentils. Additionally, the utilization of markers for biofortification holds promise in accelerating the breeding process, allowing for the selection of varieties with improved nutritional profiles while maintaining desirable agronomic traits. While significant progress has been made in crops like maize and rice, there remains untapped potential for biofortification in lentils. Continued research and investment in marker-assisted breeding and transgenic approaches are essential to unlock this potential and address the persistent challenge of malnutrition. Through collaborative efforts between researchers, breeders, policymakers, and stakeholders, biofortification initiatives can play a pivotal role in alleviating

global malnutrition and promoting food security for generations to come.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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