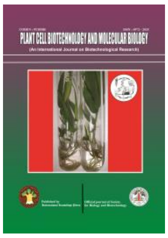

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Biofortification for Enhancement of Zinc (Zn) and Iron (Fe) Content in Wheat (*Triticum aestivum* L.): A Comprehensive Review

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Micronutrient deficiency, often termed "hidden hunger," is a significant global health issue affecting over three billion people worldwide. Given that wheat is a primary staple grain in many developing countries, it is crucial to focus on enhancing its nutritional content, as it often lacks essential micronutrients. Biofortification offers a promising solution to this problem by increasing the levels of key nutrients in the edible parts of crops through both agronomic and genetic approaches. This article examines the potential of biofortification in wheat, targeting vital micronutrients like iron and zinc. By employing traditional breeding methods alongside modern genetic techniques such as genome sequencing, Quantitative Trait Locus (QTL) mapping, and Genome Wide Association Studies (GWAS), researchers aim to boost the bioavailability and concentration of these nutrients in

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wheat varieties. The genetic variability observed in wild wheat relatives plays a significant role in identifying traits that can enhance the nutritional profile of wheat. The benefits of biofortified wheat are numerous, especially for resource-limited consumers who rely heavily on cereal-based diets. Increased concentrations of iron and zinc in wheat could improve the health and well-being of these populations, helping to combat hidden hunger on a global scale. This review paper provides a comprehensive overview of the current state of agronomic and genetic strategies for wheat biofortification, with a specific focus on increasing zinc and iron content in wheat grains. It discusses the progress made in biofortification research and outlines the various breeding approaches and genetic tools used to enhance wheat's nutritional content. By leveraging these strategies, wheat biofortification has the potential to address micronutrient deficiencies and contribute to improved public health outcomes worldwide.

Keywords: Biofortification; malnutrition; wheat; agronomic; genetic strategies.

1. INTRODUCTION

Most of the foods we consume are lacking in micronutrients, which are crucial for human development and well-being. The micronutrient deficiency or hidden hunger in developing countries, particularly in Africa and Asia is a major cause of health problems and has put an immediate financial strain on the medical system [1]. Millions of people dying each year from malnutrition, which is having detrimental effect on our growth and causing night blindness, anaemia, and weakened immunity. Over 233.9 million people in India affected by malnutrition, making it one of the biggest problems facing by the nation [2]. Zinc, folate, iodine, iron, and vitamin A deficiencies were leading causes of malnutrition. Because of the concerning rate of population growth worldwide, if immediate corrective action is not taken, the situation will worsen considerably more than anticipated in the near future [3]. Supplementation, fortification, and food diversification are possible solutions to this issue, but these tactics rely on ongoing infrastructure and investment [4]. To avoid these issues biofortification can be used, which raises the concentrations of essential micronutrients in the crops and enhance its bioavailability and uptake in the human body during digestion [5]. The process of biofortification involves enhancing the nutritional value of food crops by a variety of techniques, such as agronomic techniques, plant breeding, and genetic engineering [6].

A significant staple cereal crop, wheat (*Triticum aestivum*) accounts for at least 30% of the global intake of iron and zinc as well as 20% of calories in the diet [7, 8]. It is particular cereal thrives in cool climatic conditions and plays a significant role in ensuring nutritional and food security in India [9]. Even though wheat is one of the main cereals with the highest concentrations of micronutrients, most diets based on wheat fall short of providing the necessary amount of vital elements like zinc and iron [10]. In developing nations, severe illness is a result of the lack of these nutrients. For this reason, consuming the required levels of zinc and iron is crucial for achieving food security and putting and end to hidden hunger [11]. So, it is essential to create, release and promote appropriate biofortified wheat cultivars for widespread acceptance to ensure nutritional and food security [12. Numerous efforts have been made to address the issue by enhancing wheats genetic composition through traditional and advanced breeding methods [13]. There are different methods for biofortification of wheat (Figure 1), the agronomic technique which uses direct foliar or soli fertilizer treatment, and the conventional breeding method which involves crossing of nutrient rich varieties (wild relatives) with the varieties with desirable agronomical traits, the genomic breeding approach which uses genomic sequencing, QTL mapping, GWAS studies, and the genetic engineering techniques [14]. Under the Harvest Plus project of the CGIAR collaboration, essential nutrients are being fortified into staple crops in order to address deficits of these mineral nutrients, this is being done in partnership with both domestic and foreign partners. This approach is thought to be the most cost-effective way to address human micronutrient deficiency [15]. With the purpose of improving the Zn and Fe content of wheat, this review paper gives a thorough overview the developments in biofortification processes. It will explore about agronomic strategies, breeding methods, and genetic engineering approaches that lead to the increased concentrations of these vital micronutrients in wheat.

2. IMPACT OF ZINC (Zn) AND IRON (Fe) ON HUMAN HEALTH

Micronutrients are vital for healthy growth and development to proceed. These constituents are essential as they serve as the structural element

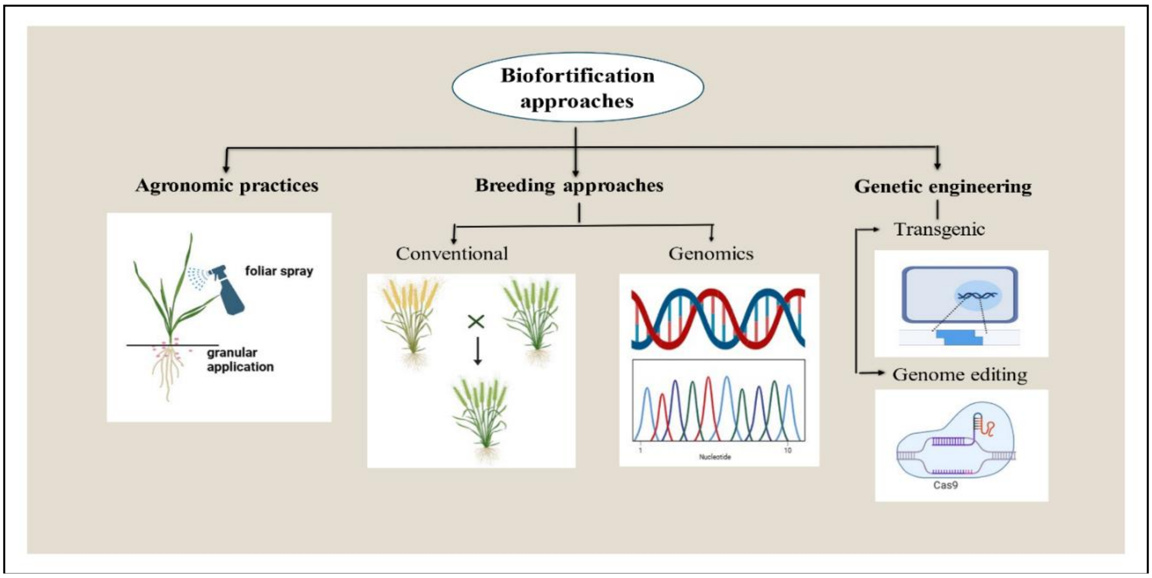

Fig. 1. Methods of wheat biofortification

or cofactor for numerous proteins and enzymes involved in diverse physiological and biological processes [16]. Human growth and development may be negatively impacted if any of these micronutrients impacted if any of these micronutrients are lacking. Around 2.5 billion people worldwide are zinc deficient [17]. Because it functions as a catalytic, structural, and signalling component for multiple proteins responsible for cell proliferation, neuronal development, immunity, signal transduction, migration, survival, and death in the human body, Zn, the most prevalent trace element, is essential to many biochemical processes which influence growth and development [18]. Additionally, zinc is a component of several enzymes and protein receptors that are found in hormones such as steroids, thyroid, and vitamins A and D [10]. For the generation and breakdown of some products of metabolism, like proteins, lipids, carbohydrates, and nucleic acids, a range of enzymes, including isomerases, hydrolases, oxidoreductases, ligases and lyases, with a large Zn fraction are needed. But excessive Zn consumption might also be harmful [19].

Iron (Fe), another significant trace metal, is similarly in charge of many bodily processes [20]. Worldwide, iron deficiency affects over two billion individuals, and it continues to be the leading cause of anaemia globally [21]. The majority of the protein in red blood cells called haemoglobin, which carries oxygen from lungs to every tissue in the body is made up of iron [22]. For the development of brain and cognitive function, iron is essential, especially during childhood. Additionally, Fe is necessary for DNA synthesis, cell division, immunological function, and also it aids in maintaining a healthy body temperature [8]. Chronic renal illness, inflammatory bowel disease, cancer, and heart failure are all brought on by a deficit in Fe. But on the other hand, hemochromatosis, which is typified by iron buildup in liver that results in fibrosis, is brought on by excessive iron ingestion [23].

3. AGRONOMIC BIOFORTIFICATION OF WHEAT

Agronomic biofortification is a typical agricultural practice used to alleviate hidden hunger and boost the content of micronutrients in grains [24]. Applying micronutrients (fertilizers) directly to the soil or plants is a viable method for overcoming malnutrition in underdeveloped nations, and it may also increase the production and quality of the grains of wheat [25], but it is a temporary solution. Plant-available zinc content in approximately 50% of the world's wheat grown soil is deemed inadequate. Because of these circumstances, wheat cultivars are unable to fully absorb and accumulate zinc, which further lowers grain zinc concentrations. Zn severs as a vital element and plays multiple structural and functional roles as an enzyme catalyst [26]. Depending on the severity of zinc shortage, this results in yield losses and low Zn concentrations in grain. Consequently, in order to ensure that roots are able to absorb zinc in sufficient amounts under these circumstances, zinc fertilizer application to the soil is essential [27].

Several field tests involving different soil and foliar fertilizer applications on wheat have been conducted during the past seven to eight years in 12 countries under the HarvestZinc project [28]. It is evident that foliar zinc sprays were very successful in raising grain zinc content in field conditions, whereas soil zinc applications at the time of sowing had minimal impact [29]. For processes including chlorophyll production, respiration, and photosynthesis, iron is essential to plant growth and development [30]. It has been reported that plants cultivated in soils poor in iron have low levels of iron in their edible parts, which eventually leads to iron insufficiency in humans [31]. To increase wheat crop productivity while maintaining high grain quality, foliar spraying a combination of 0.5% ZnSo4 and 1% FeSo4 is advised [24].

4. CONVENTIONAL BREEDING FOR BIOFORTIFICATION OF WHEAT

Breeding wheat for increased nutritional content is one long-term solution to the problem of micronutrient shortage in developing countries [32]. Here, in order to develop plants with appropriate nutrient contents and agronomic qualities, parental lines with high levels of nutrients are crossed with the lines possessing targeted agronomic characteristics over a number of generations [33]. Wheat biofortification has benefited greatly from backcross breeding or

Table 1. List of wheat cultivars rich in Zn and Fe developed in India [36]

S. No	Variety	Nutrient content	Institution	Year of release
1.	WB 02	Zn – 42.0 ppm, Fe – 40.0 ppm	ICAR – Indian Institute of Wheat and Barley Research, Karnal	2017
2.	HPBW 01	Fe – 40.0 ppm, Zn – 40.6 ppm	Punjab Agricultural university (PAU), Ludhiana	
3.	HD3171	High Zn – 47.1 ppm	ICAR – IARI, New Delhi	
4.	HD 1605	Fe – 43.0 ppm	ICAR – IARI, Indore	
5.	HI 8759	Zn – 42.8 ppm Fe – 41.1 ppm	ICAR – IARI, Regional Station, Indore	
6.	PBW 757	High Zn – 42.3 ppm	PAU , Ludhiana	2018
7.	MACS 4028	Zn – 40.3 ppm, Fe – 46.1 ppm	Agharkar Research Institute, Pune, India	
8.	HI 8777	Zn – 43.6 ppm, high Fe – 48.7 ppm	ICAR – IARI, Indore	
9.	DBW 173	Fe – 40.7 ppm	ICAR – Indian Institute of Wheat and Barley Research, Karnal	
10.	DBW 187	Fe – 43.1 ppm	ICAR – Indian Institute of Wheat and Barley Research, Karnal	2018 & 2020
11.	HI 1633	High Fe – 41.6ppm, Zn – 41.1 ppm	ICAR – IARI, Indore	2020
12.	HD 3298	Fe – 43.1 ppm	ICAR – IARI, New Delhi	
13.	PBW 771	Zn – 41.4 ppm	PAU, Ludhiana	
14.	HUW 711	Zn	Banaras Hindu University, India	
15.	H1 8805	Fe – 40.4 ppm	ICAR – IARI, Indore	
16.	HD 1605	Fe – more than 43 ppm)	—	
17.	HD 3249	Fe – 42.5 ppm)	ICAR – IARI, New Delhi	
18.	DDW 47	Fe – 40.1 ppm)	ICAR – Indian Institute of Wheat and Barley Research, Karnal	

Table 2. QTLs identified for grain Zn and Fe content in wheat

Trait	Mapping population	Name or no. of QTLs	References
Zinc (Zn)	DH	11QTLs	[52]
	DH	4QTLs	[53]
	RILs	2QTLs	[54]
	RILs	6QTLs	[55]
	RILs	2QTLs	[56]
	RILs	2QTLs	[57]
	RILs	1QTL	[58]
	RILs	3QTLs	[59]
	RILs	3QTLs	[60]
	RILs	3QTLs	[61]
	DH	2QTLs	[62]
	RILs	6QTLs	[63]
	RILs	2QTLs	[64]
	RILs	2QTLs	[65]
	RILs	3QTLs	[66]
	RILs	2QTLs	[67]
	RILs	2QTLs	[68]
Iron (Fe)	DH	1QTLs	[53]
	RILs	3QTLs	[54]
	RILs	11QTLs	[55]
	RILs	2QTLs	[56]
	RILs	3QTLs	[57]
	DH	4QTLs	[63]
	RILs	1QTL	[52]
	RILs	3QTLs	[54]
	RILs	3QTLs	[55]
	DH	1QTL	[56]
	RILs	4QTLs	[57]
	RILs	2QTLs	[58]
	RILs	1QTL	[59]
	RILs	3QTLs	[59]
	RILs	2QTLs	[61]
	RILs	2QTLs	[62]

heterosis breeding [34-36] and participatory varietal selection (PVS). Generally, genetic variation for grain Fe and Zn content is lower in breeding lines and cultivars than in landraces, cultivated wheat progenitors, and related wild species such as *Triticum turgidum* ssp. *diccocoides*, *T. turgidum* ssp. *Dicoccum*, *T. aestivum* spp. *Spelta,* and *Aegilops tauschii* [34]. Breeding wheat for improved nutritional quality by increasing micronutrient concentrations was started as part of the HarvestPlus programme by crossing rich zinc and iron sources found in *T. spleta*, landraces from Mexico and Iran, and synthetic wheats [10]. In south Asia, these crosses have produced wheat lines with competitive yields and improved grain zinc content as a result of international organisations collaboration with national research institutes [34]. The last few years seen a surge in the production of wheat cultivars high in zinc and iron in India. Five research institutes generated eleven bread wheat varieties and five durum wheat types which are approved by the Indian government's central variety release committee were rich in zinc and iron, or both (Table 1). Zinc rich varieties called Zincol 2016, Akbar 2019, Nawab [23] has been released in Pakistan [33].

5. GENOMIC APPROACHES FOR BIOFORTIFICATION OF WHEAT

Utilising the genetic information found in the genome of the plant, genomic techniques for wheat biofortification aim to create wheat varieties with higher concentrations of vital minerals like zinc and iron [37]. These methods target certain genes or genetic pathways that are in charge of nutrient intake, transport, and accumulation with accuracy and efficiency [38]. Utilising genetic mapping techniques such as QTL mapping employing different populations and GWAS, unique genomic areas and genes controlling significant nutrient aspects of wheat can be found [39]. This will improve effectiveness of biofortification breeding programmes and make it easier to design and introduce DNA markers into superior genetic lines [10].

5.1 Genome Sequencing and Annotation

For the wheat landrace Chinese Spring reference, several genome assemblies have been created, varying in terms of completeness and annotation [43-46]. This genome assembly's high proximity will aid in the genetic mapping of loci linked to the accumulation of iron and zinc. Annotation of 107891 highly accurate gene models will help in interpret loci found in GWAS and QTL studies and enable genome-wide exploration of gene families linked to biofortification [40]. Additional cultivars are presently undergoing high quality genome sequencing and annotation, which will facilitate independent validation of gene models and enable comparison [8]. Hundreds of wheat lines exotic regions have been sequenced by the use of exome captures in studies [47-48], this technique may be utilised to find unique diversity among genes that have been suggested for biofortification. According to [32], wild progenitor species often possess high concentrations of iron and zinc and may have genes that are absent in hexaploidy wheat. Furthermore, several wild cultivar accessions are being sequenced in an attempt to uncover further genetic diversity.

5.2 Quantitative Trait Loci (QTL) Mapping

Using QTL mapping, areas of the wheat genome linked to nutrient-related characteristics, like zinc and iron accumulation in grains can be found. Then, using markers associated with these QTLs, wheat varieties with enhanced biofortification traits can be bred using MAS [10, 49]. To map QTLs for biofortification attributes, a variety of mapping populations have been utilised, including doubled haploid lines (DH), F2 derived F3 or F4, BC3F2:3, BC5F2:6,) or single seed descent lines (SSDs) recombinant inbred lines (RILs) [50]. In 1977, a RIL population resulting from a hybrid between wild emmer (*Triticum turgidum*) and *duram* wheat on chromosome 6BS was shown to harbour the first QTL for zinc as well as iron concentration in wheat [51]. Numerous QTL studies (Table 2) that have been conducted to investigate the genomic areas for iron and zinc levels have discovered several number of QTLs for wheat grain Fe and Zn content. In superior genetic lines, these lines can be utilised to improve the micronutrient contents.

5.3 Genome Wide Association Studies (GWAS)

The QTL resolution and allele coverage of the genomic areas found using GWAS are good because they capture variation that occurs across the germplasm such as landraces, introduced varieties and improved breeding lines that constitute a broad gene pool [69,70]. For wheat biofortification, (GWAS) is an essential method for detecting genetic variants linked to characteristics pertaining to wheat's nutrient content, such as increased concentrations of minerals like iron, zinc [10]. Numerous research using GWAS have been conducted globally to determine MTA for the Zn and Fe content of wheat [71-79].

5.4 Genetic Engineering for Wheat Biofortification

Genetic engineering is another method for developing nutrient-enriched cultivars of crops. This methos involves utilising transgenic technology to directly insert a desired gene into a superior variety, for increasing the amount of micronutrients [80]. Utilising genetic engineering to boost wheats Zn and Fe content has not been attempted very often. [81] used the wheat ferritin gene (TaFer1-A) in an attempt to raise the Fe content in the grain of wheat, and the overexpression of the ferritin gene increased the Fe content of the grain by 50-80%. In the endosperm of wheat grains, a recent study found that overexpression of the VIT gene using an endosperm-specific promoter increased the amount of iron in the white four fractions by two times without increasing the amount of the antinutrient phytate [82]. Wheat grain zinc and iron bioavailability is enhanced by CRISPR-Cas-mediated lowering of phytic acid levels via inositol pentakisphosphate 2-kinase 1 (TaIPK1) disruption [83].

6. FUTURE DIRECTIONS

A growing global concern for humans is malnutrition or micronutrient deficiency [84]. Therefore, the most dependable, practical, and affordable method of providing micronutrients to crops with low nutrient population is biofortification. Due its widespread use in developing nations and its status as the primary staple crop in temperate regions, wheat has to be biofortified with micronutrients, particularly Zn and Fe to satisfy the necessities for improved human well-being [62]. The amount of these vital micronutrients in wheat grains have significantly increased thanks to agronomical techniques and advances in conventional and advanced breeding techniques. Various stress caused decline in nutrients content [85-86]. Biofortified wheat has shown in numerous studies to be beneficial in enhancing the nutritional status and health consequences of people that are zinc and iron deficient. In future, maintaining significant amounts of Zn and Fe in the different environmental conditions and genetic backgrounds is needed. In order to ensure that the human body can properly absorb and utilise iron and zinc, it is equally vital to improve the quality of these nutrients in wheat and maize [87] grains as well as their bioavailability. Therefore, to provide complete nutritional advantages, future research should investigate the viability and agronomic consequences of breeding wheat varieties that are biofortified with numerous micronutrients. Additionally, the development of biofortified wheat varieties may proceed more quickly if there is coordination and collaboration amongst experts from various disciplines including plant breeders, seed technologists, agronomists, biotechnologists, biochemists, and postharvest technologists—across various private and public organisations.

Future aspects for biofortification of wheat to enhance zinc (Zn) and iron (Fe) content hold significant potential for improving global nutrition and combating hidden hunger. As the world's population grows and dietary patterns shift, biofortification offers a sustainable solution to address micronutrient deficiencies. Research in this field will likely focus on leveraging advanced genetic tools and breeding techniques to further enhance the micronutrient content in wheat. Techniques such as genome sequencing, CRISPR-based genome editing, and Quantitative Trait Locus (QTL) mapping will play a crucial role in identifying and manipulating the genes responsible for higher Zn and Fe concentrations. These advancements will enable the development of wheat varieties that are not only nutritionally superior but also adapted to diverse climatic conditions and resistant to pests and diseases. Another key area for future research is the exploration of wild relatives of wheat, which often possess higher levels of essential micronutrients. These wild relatives can provide a valuable genetic pool for enhancing the nutritional profile of cultivated wheat. By studying the genetic makeup of these wild species, researchers can identify the specific genes responsible for increased Zn and Fe content, allowing for more targeted breeding approaches. Furthermore, the integration of bioinformatics and big data analytics into breeding programs will facilitate the rapid identification of desirable traits and accelerate the development of biofortified wheat varieties. Beyond the scientific and technical aspects, the future of biofortification in wheat must also consider the socio-economic impact and the acceptance of biofortified crops by consumers. Public awareness and education about the benefits of biofortified wheat will be essential to ensure its widespread adoption. Additionally, partnerships between researchers, policymakers, and agricultural stakeholders will play a pivotal role in promoting the cultivation and distribution of biofortified wheat, contributing to a more sustainable and nutritionally secure future.

7. CONCLUSION

Hidden hunger remains a critical global health issue, affecting billions of people, particularly in developing countries where wheat is a staple food. Given wheat's central role in global diets, biofortification presents a viable solution to address the lack of essential micronutrients, specifically iron and zinc. Through a combination of agronomic practices and modern genetic techniques, such as genome sequencing, Quantitative Trait Locus (QTL) mapping, and Genome Wide Association Studies (GWAS), researchers can significantly improve the bioavailability and concentration of these key nutrients in wheat varieties. The use of genetic variability from wild wheat relatives offers a robust source of traits that can be incorporated into cultivated wheat to enhance its nutritional profile. This approach not only supports increased micronutrient content but also aligns with sustainable agricultural practices. Biofortified wheat has the potential to provide significant health benefits, particularly for populations reliant on cereal-based diets, improving their nutritional intake and combatting hidden hunger. This review underscores the current progress in wheat biofortification, outlining the various breeding approaches and genetic tools that have been successful in enhancing zinc and iron content. By adopting these strategies, the agricultural community can contribute to improved food security and public health, offering a sustainable path forward to address global micronutrient deficiencies. The future of biofortification in wheat holds promise for significant advancements in public health and the fight against global malnutrition, providing a brighter and healthier future for many.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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