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ENHANCING THE NUTRITIONAL QUALITY OF WHEAT: A REVIEW OF AGRONOMIC AND GENETIC BIOFORTIFICATION APPROACHES

Original Article

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ABSTRACT

Wheat (*Triticum aestivum* L.) is a globally important cereal crop, serving as a dietary staple for much of the world's population. However, modern wheat varieties are often deficient in micronutrients such as iron (Fe) and zinc (Zn), contributing to widespread malnutrition, particularly in developing regions. To address this issue, two main strategies have been adopted: agronomic and genetic biofortification. Agronomic biofortification involves the application of fertilizers to soil or foliage to temporarily boost nutrient content in the grain. While effective in the short term, this approach requires continuous input and can be influenced by environmental conditions. In contrast, genetic biofortification aims to develop wheat cultivars that naturally accumulate higher levels of Fe and Zn through conventional breeding and modern techniques such as QTL mapping and marker-assisted selection (MAS). This method offers a more sustainable and long-lasting solution. Researchers are also exploring high-mineral landraces, wild relatives, and colored wheat varieties for their added nutritional value. Combining both agronomic and genetic strategies may provide the most effective pathway to combat micronutrient deficiencies. Future research should focus on improving breeding efficiency, exploiting underutilized germplasm, and enhancing nutrient uptake to develop nutritionally enriched wheat varieties and support global food security.

Keywords: Wheat (*Triticum aestivum* L.), Micronutrients, Iron (Fe), Zinc (Zn), Biofortification, Agronomic biofortification, Genetic biofortification, Wheat and Hidden Hunger

INTRODUCTION

Wheat (Triticum aestivum L.) is one of the most important cereal crops globally, forming the dietary foundation for more than a third of the world's population (Mohammadi-joo et al., 2015). It thrives in diverse agro-ecological zones, especially in dry and semi-arid environments, due to its long-day photoperiodic response, self-pollination, and adaptability to fluctuating climatic conditions (Belderok, 2000). The predominance of hexaploid bread wheat in global agriculture accounting for nearly 95% of wheat production underscores its critical role in food systems, particularly in the manufacture of bread and other processed foods (Debasis and Khurana, 2001). As a cornerstone of global food security, wheat's production trends, nutritional value, and adaptation to agronomic advancements have profound implications for both developed and developing regions. Recent decades have seen a substantial increase in global wheat output, culminating in a record-high production of 765.41 million metric tons in 2020. This upward trajectory is attributed to a convergence of factors, including the development of high-yielding, climate-resilient cultivars, adoption of precision farming practices, optimized fertilizer regimes, and broader access to biofertilizers and irrigation technologies. Furthermore, increasing consumer demand particularly in urban and emerging markets for wheat-based products has driven the need for enhanced production efficiency and area expansion. Between the 2004/05 and 2010/11 seasons, global wheat production and consumption remained relatively stable, averaging between 600–650 million metric tons (Mmt). Minor annual variations during this period were largely influenced by regional climatic challenges such as droughts and unseasonal rainfall, particularly in major wheat-producing nations like Russia, Canada, and the United States. These disruptions also coincided with logistical challenges and geopolitical uncertainties, which led to fluctuations in grain availability and global price volatility. Despite these adversities, the wheat sector showed remarkable resilience, maintaining consistent production and consumption levels. A notable surge in both production and consumption occurred from 2011/12 through 2016/17. By the 2013/14 season, global wheat production had surpassed 700 Mmt, reflecting the cumulative impact of innovations in crop breeding, pest and disease management, and government-led food security initiatives. During this time, countries such as China, India, and Russia significantly increased wheat output by expanding cultivated areas, adopting improved genotypes, and investing in research and development. Consumption mirrored this growth, driven by dietary transitions in Asia and Africa, where rising urbanization and disposable incomes shifted consumer preferences toward wheat-based foods like bread, pasta, and noodles.

The years spanning 2017/18 to 2021/22 marked an unprecedented phase in wheat production, with yields approaching 800 Mmt during the 2020/21 season. This remarkable performance was enabled by synergistic factors such as favorable agro-climatic conditions, rapid adoption of digital agriculture and smart farming tools, and enhanced mechanization across wheat belts. Simultaneously, consumption patterns intensified, driven not only by direct human intake but also by secondary uses, including wheat's growing role in livestock feed especially in China. Population growth, rising incomes, and expanded food processing sectors in developing countries further contributed to this upward demand trend. While occasional disruptions such as trade tensions, political instability, and climate anomalies did affect supply chains, the overall market balance between production and consumption remained steady. However, this progress in wheat quantity contrasts sharply with concerns about its nutritional quality. Most commercial wheat cultivars, though high yielding, are inherently low in essential micronutrients such as iron (Fe) and zinc (Zn), which are vital for human health (Welch and Graham, 2004). Moreover, post-harvest processing especially refining and milling often strips wheat of its nutrient-rich outer layers, resulting in flour products that are significantly depleted in micronutrients. This problem is especially concerning in regions where wheat serves as the primary staple, as reliance on nutrient-poor diets has contributed to widespread deficiencies. Globally, over two billion people suffer from micronutrient malnutrition, with Asia and sub-Saharan Africa bearing the highest burden (Grew, 2018). Efforts to combat these deficiencies have led to the emergence of biofortification as a sustainable, food-based strategy. Biofortification refers to the enhancement of a crop's nutritional profile through agricultural or genetic means. Agronomic biofortification involves the application of mineral fertilizers either to soil or foliage to raise nutrient concentrations in edible plant parts. While effective in the short term, this approach requires repeated inputs and may be limited by soil properties, environmental factors, and economic accessibility (Fageria et al., 2002; Fageria and Baligar, 2008). Genetic or genomic biofortification, on the other hand, offers a more durable solution by enhancing the crop's intrinsic ability to uptake, transport, and accumulate micronutrients. This approach involves breeding wheat varieties with higher grain Fe and Zn content using both conventional and molecular tools. Recent advances in plant genomics such as QTL mapping, Marker-Assisted Selection (MAS), and genomic selection have allowed researchers to target polygenic traits responsible for micronutrient accumulation (Ahmadi et al., 2018). Unlike monogenic traits, traits related to micronutrient content are complex and influenced by both genetic and environmental interactions, making their improvement a significant challenge. Nonetheless, progress has been made through the identification of favorable alleles, utilization of high-mineral germplasm, and development of biofortified lines with improved mineral uptake efficiency (Breseghello and Coelho, 2013; Cui et al., 2015; Moose and Mumm, 2008).

An integrated biofortification strategy combining both agronomic and genetic approaches may offer the most effective and scalable solution. While agronomic interventions can serve as short term measures to alleviate deficiencies, especially in vulnerable populations, genetic improvements provide a foundation for sustained nutritional enhancement without the need for recurrent external inputs. In the context of global food security and health, investing in biofortified wheat cultivars represents a vital step toward reducing hidden hunger and improving dietary quality. This review aims to explore the evolution and interconnection of wheat production dynamics and nutritional challenges. Specifically, it seeks to identify effective biofortification methods that enhance iron and zinc content in wheat while addressing the growing demands of global food systems. By evaluating current research, breeding strategies, and agronomic innovations, this study highlights the need for integrated, sustainable solutions to secure both the quantity and quality of future wheat harvests.

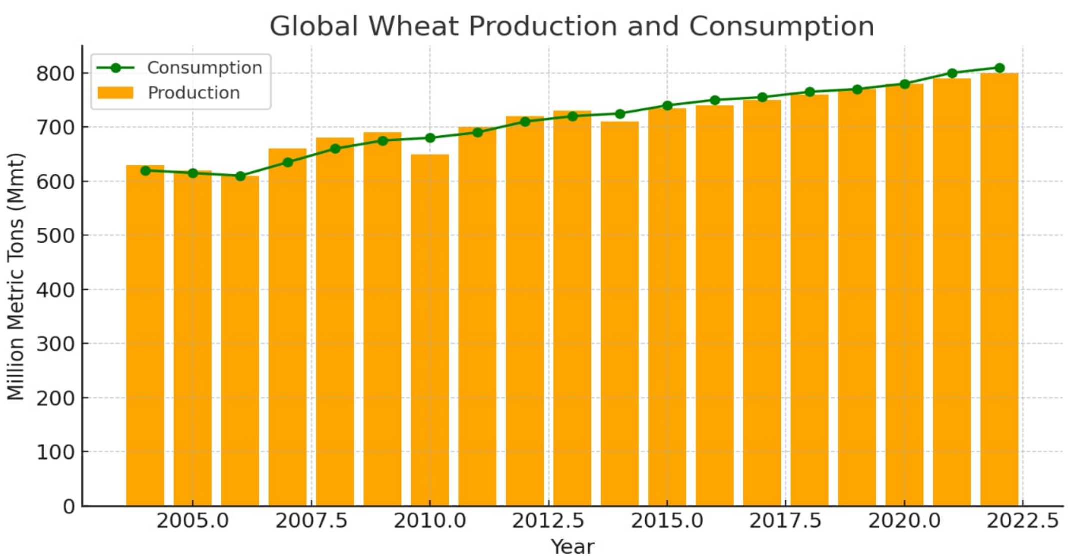

Figure 1 Global wheat production from 2005-2022 source USDA (2022) Global Wheat Production and Consumption (2004/05 - 2021/22)

BIOFORTIFICATION FOR ENHANCING FE & ZN CONTENT

For proper metabolic functioning, the human body depends on a steady intake of essential minerals and micronutrients, most of which are acquired through daily food consumption. However, staple crops like wheat often fall short in providing adequate levels of critical micronutrients, particularly iron (Fe) and zinc (Zn). This shortfall contributes significantly to "hidden hunger," a form of chronic micronutrient deficiency prevalent in low-income communities across the developing world. Inadequate intake of Fe and Zn is associated with numerous adverse health outcomes, including delayed physical growth, compromised immune response, diminished work capacity, and developmental issues in children (Tulchinsky, 2010).

Although immediate interventions such as dietary supplementation, food fortification, and diversification can temporarily alleviate micronutrient deficiencies, they are often limited by cost and accessibility. In contrast, biofortification has emerged as a more sustainable and economically feasible solution. This approach aims to enhance the micronutrient content of wheat directly, thereby reducing the need for external fortification after harvest (Ruel and Alderman, 2013). Two major pathways have been developed to achieve biofortification: genetic approaches that involve breeding wheat varieties with naturally higher micronutrient concentrations, and agronomic techniques that apply micronutrient-rich fertilizers to the soil or foliage and also seed priming (Mara and Petra, 2012; White and Broadley, 2009). These methods improve the inherent nutritional quality of wheat and offer a promising long-term strategy to reduce iron and zinc deficiencies. Figure 2 provides a visual summary of these biofortification techniques, detailing the mechanisms used to elevate Fe and Zn content in wheat grains.

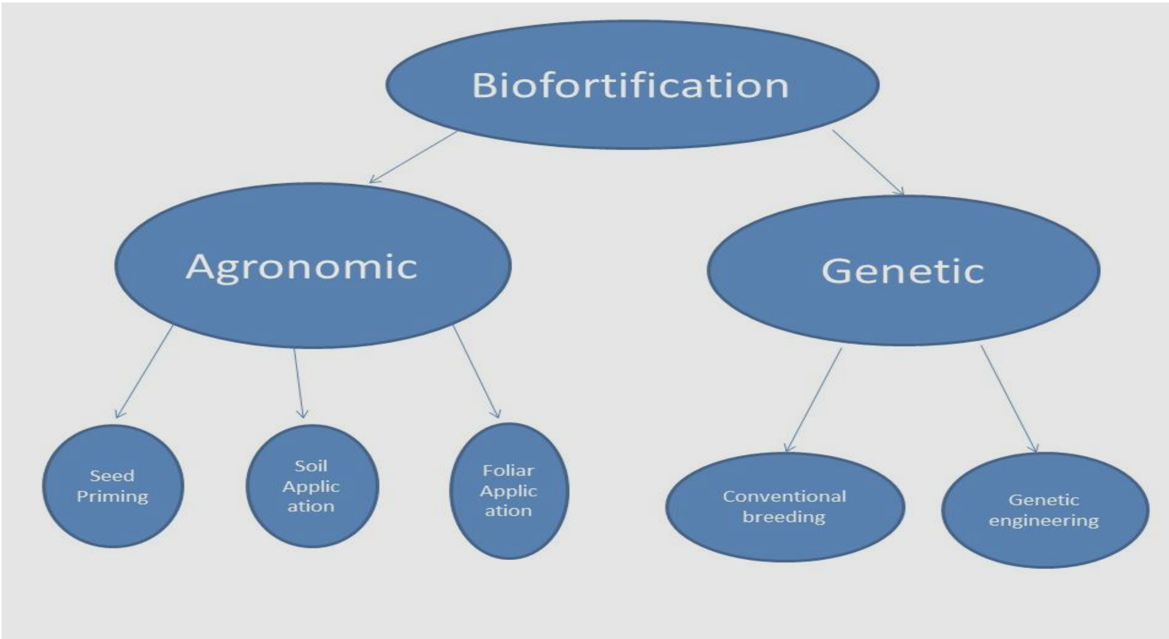

Figure 2 Classification of biofortification in wheat

AGRONOMIC BIOFORTIFICATION

In countries like Pakistan, where agricultural soils particularly those used for wheat and other cereal crops often suffer from physical and chemical deficiencies, the availability of essential micronutrients such as iron (Fe) and zinc (Zn) to plants is severely limited. Under these circumstances, agronomic biofortification emerges as a practical intervention, involving the application of micronutrient-rich fertilizers either directly to the soil or through foliar spraying onto plant leaves (De Valença *et al*., 2017). The use of fertilizers to enrich food crops with essential minerals has long been a part of agricultural practices, aiming to enhance nutrient uptake and ultimately improve the nutritional composition of edible plant parts (Rengel *et al*., 1999). The effectiveness of agronomic biofortification can vary depending on the method of fertilizer application. Both foliar and soil-based treatments influence not only the concentration of Fe and Zn in the grains but also overall crop yield. Optimizing the timing and method of fertilizer delivery—whether to plant leaves or soil—is essential for maximizing micronutrient accumulation in grains. The pathway of micronutrients from soil to human consumption involves several steps, beginning with root uptake, internal plant distribution, remobilization to the grain, and finally, bioavailability and absorption within the human body. Factors such as soil pH, organic matter content, aeration, moisture, and nutrient interactions all influence how readily micronutrients are available for plant uptake (Alloway, 2009). Although soil-based applications are common, foliar feeding is sometimes preferred for its rapid nutrient delivery and cost-effectiveness, particularly when used to correct deficiencies identified through visual symptoms or plant tissue analysis (Cakmak *et al*., 2010; Zou *et al*., 2010; Peleg *et al*., 2007; Li *et al*., 2016). Soil applications, in contrast, are typically guided by soil testing results. Agronomic biofortification, while widely practiced as a short-term approach to addressing micronutrient deficiencies, has several limitations compared to genetic strategies (Cakmak, 2008.). One major drawback is the inconsistency in nutrient translocation from vegetative tissues to seeds, which may prevent the nutrients from reaching the edible portions of the crop (Frossard *et al*., 2000). Furthermore, the repeated need for fertilizer applications raises concerns about long-term economic feasibility and environmental impact, making agronomic methods less sustainable than genetic biofortification (Singh *et al*., 2016). Despite these challenges, agronomic biofortification particularly through foliar feeding remains an effective interim solution for combating Fe and Zn deficiencies in wheat. It ensures that essential nutrients are delivered to the parts of the plant that are consumed, even in soils with limited micronutrient availability. Figure 3 provides an overview of both foliar and soil-based fertilizer application methods used in wheat production.

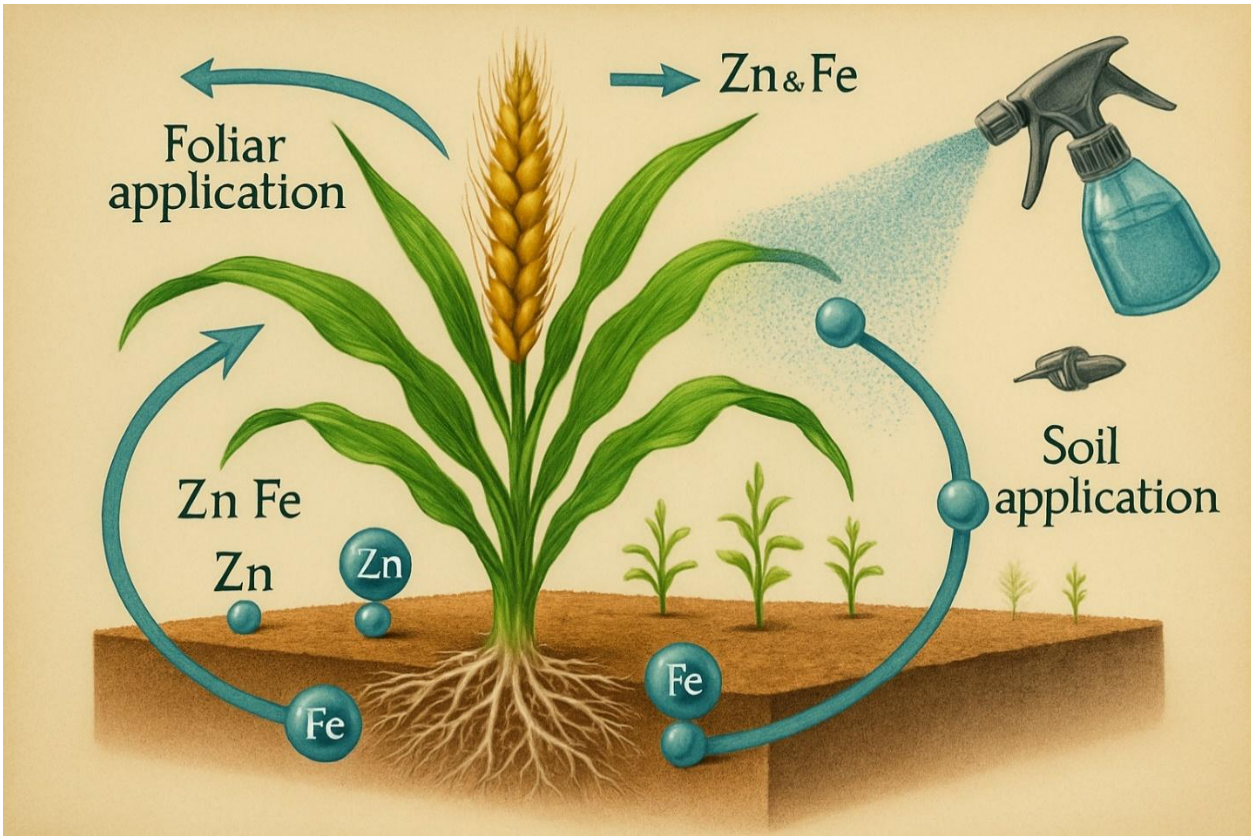

Figure 3 Agronomic biofortification (Foliar and soil application) in wheat

GENETIC BIOFORTIFICATION:

Genetic biofortification refers to the enhancement of nutrient concentration and bioavailability in crops through the use of traditional plant breeding methods and modern genetic technologies, including recombinant DNA approaches. This strategy capitalizes on the existing genetic diversity related to nutrient density, aiming to improve nutrient accumulation in edible plant parts via gene discovery, marker-assisted selection, and conventional breeding practices (Grusak, 2002). The ultimate goal is to develop crop varieties particularly wheat that exhibit improved uptake and transport of minerals such as iron (Fe) and zinc (Zn), enhanced nutrient deposition in the grain, especially the endosperm, reduced levels of anti-nutritional factors, and greater bioavailability for human consumption (Mulualem, 2015). Breeding institutions and researchers are working to create wheat lines enriched with iron, zinc, and compounds that support nutrient absorption. Although modern wheat cultivars generally show limited variation in mineral content, their wild relatives offer a rich genetic reservoir for improving grain nutrition (White and Broadley, 2009). Investigations into ancient varieties, landraces, and wild wheat accessions have demonstrated that wild relatives may possess up to three to four times higher levels of Fe and Zn compared to widely cultivated modern varieties (Rawat *et al*., 2009). Moreover, the physiological and genetic traits linked to iron and zinc accumulation appear to be consistent across wild, spelt, and modern wheat types (Cakmak *et al*., 2004; Morgounov *et al*., 2007; Gomez-Becerra *et al*., 2010b).To incorporate desirable traits such as improved yield, stress resistance, and nutritional quality, breeders frequently utilize wild species in crossbreeding programs. Genes responsible for enhanced mineral content in grains can be transferred from wild relatives using both traditional crossbreeding and modern biotechnological tools (Chhuenja *et al*., 2008). Unlike conventional methods, genetic engineering allows for the introduction of beneficial genes from non-sexually compatible species or even synthetically created genes expanding the scope of trait integration. Traditional breeding, by contrast, is limited to transferring genes from compatible donor plants (Singh et al., 2016). Advancements in this area have shown substantial progress in mitigating micronutrient deficiencies, positioning genetic biofortification as a long-term and sustainable strategy to combat global malnutrition (Ludwig and Slamet-Wedin, 2019). One of the major benefits of this approach is its cost-efficiency; once nutrient-enriched varieties are developed during the research and development (R&D) phase, they can be distributed and cultivated with minimal recurring investment (Singh *et al*., 2016). As noted by Saini *et al*. (2020), ongoing innovation in genetic biofortification holds great potential for delivering sustainable nutritional improvements in staple crops, thereby addressing widespread micronutrient deficiencies on a global scale.

PLANT BREEDING APPROACHES

Over the past five decades, wheat breeding has largely centered on improving traits related to yield and adaptability, such as plant stature, resistance to diseases, higher harvest index, and greater biomass production (Ortiz *et al*., 2007). These improvements have relied heavily on exploiting the genetic diversity associated with such agronomic traits, which remains fundamental to crop enhancement through breeding (Ortiz *et al*., 2007). Alongside yield enhancement, the nutritional quality of wheat grain has become increasingly important to meet the dietary needs of the expanding global population. Despite its status as a staple crop, wheat contains relatively low levels of bioavailable iron (Fe) and zinc (Zn), with only around 5% and 25% of each nutrient available from the grain, respectively. As a result, improving the nutritional content and bioavailability of these micronutrients is an emerging priority in modern breeding strategies. Fe and Zn concentrations in wheat grain are quantitatively inherited, as established in prior research (Trethowan *et al*., 2005; Trethowan *et al*., 2007). Unlike qualitative traits, which are typically governed by single genes, quantitative traits like mineral content are influenced by multiple genes and environmental interactions, making them more complex targets for breeders. Nevertheless, specific genotypes including landraces, wild relatives, and early-stage lines from biofortification initiatives have demonstrated significantly higher Fe and Zn content compared to most commercial cultivars, indicating strong potential for nutritional improvement (Monasterio and Gresham, 2000). The International Maize and Wheat Improvement Center (CIMMYT) has been at the fore front of genetic biofortification by incorporating genes from nutrient-rich species such as *Triticum aestivum* ssp. *spelta* and *Triticum turgidum* ssp. *dicoccon* into elite wheat varieties. This approach aims to combine high yields with enhanced grain nutrition (Velu *et al*., 2013). Moreover, pigmented wheat varieties such as purple, blue, and black wheat have attracted global breeding interest due to their elevated phenolic content and potential health benefits. Several of these colored wheat lines are now in commercial production (Garg *et al*., 2018; Shao *et al*., 2011; Sharma *et al*., 2018). As emphasized by Pleiffer and McClafferty (2007), breeding pipelines should be driven by their potential nutritional impact, with biofortification positioned as a core objective. These pipelines must consider key nutritional factors, including population-specific micronutrient demands, nutrient bioavailability, and the stability of micronutrient levels after food processing, storage, and cooking (Cakmak *et al*., 2010; Pleiffer and McClafferty, 2007). The integration of modern molecular tools such as Quantitative Trait Locus (QTL) analysis and Marker-Assisted Selection (MAS) has facilitated the identification and incorporation of high-mineral traits in breeding programs. These tools also account for environmental variables, including soil characteristics like pH and organic matter content, which can significantly influence the uptake and accumulation of micronutrients in wheat grains (Saini *et al*., 2020).

CONVENTIONAL PLANT BREEDING

In recent decades, global wheat grain yields have shown a consistent upward trend, with much of this improvement credited to advancements in crop varieties. Across many regions, wheat production still largely depends on conventional breeding techniques, which have evolved over centuries to improve crop performance. These traditional approaches focus on altering the genetic makeup of wheat to develop varieties capable of absorbing and storing higher levels of bioavailable micronutrients. One of the key benefits of such nutritionally improved crops is their long-term impact after the initial research and breeding investment, the resulting varieties can continue to deliver nutritional benefits with relatively low ongoing costs (Gomez-Galera *et al*., 2010). In particular, conventional plant breeding offers an effective path for improving agronomic characteristics, grain quality, and micronutrient density in wheat, especially in regions where smallholder and subsistence farming dominates (Singh et al., 2016). Extensive efforts have been undertaken to exploit natural genetic variation in mineral content across different wheat germplasms using conventional selection methods (Qaim *et al*., 2007). A range of breeding strategies has been applied to develop nutrient-rich genotypes, including techniques suitable for both vegetative crops and species that propagate via cross- or self-pollination (Gupta *et al*., 2010). For self-pollinating crops such as wheat, breeders often use pure line or mass selection to isolate superior genotypes, while cross-pollinated species benefit more from recurrent selection or population improvement methods to maintain and enhance genetic diversity (Gupta *et al*., 2010). However, in cases where existing genetic variation is insufficient to achieve desired nutrient levels, breeders may need to turn to genetic engineering for further enhancement. Conventional breeding primarily works within the scope of existing gene pools, occasionally incorporating genes from wild relatives that can be successfully crossed with cultivated wheat. The most frequently used strategy for improving nutrient content in wheat involves introgression of beneficial traits from distant relatives into commercial lines. However, this process can be slow and resource-intensive, and in some situations, it may prove impractical or inefficient. Despite its broad application, conventional breeding has notable limitations. Because it often depends on phenotypic evaluation or visual selection, the process tends to be time-consuming, laborious, and susceptible to inaccuracies. These limitations can introduce significant variability and potential error into selection outcomes, reducing overall breeding efficiency (Mwandaingoni *et al*., 2017).

MUTATION BREEDING APPROACHES

Mutation breeding has played a significant role in enhancing a wide range of crops, including wheat, by introducing beneficial genetic variations that support the development of improved agronomic traits. This approach has been extensively applied in both industrialized and developing nations to produce grain varieties with superior quality, higher yields, and other targeted improvements (Singh *et al*., 2016). Induced mutations, created through the use of mutagens, have proven particularly effective in increasing wheat productivity by improving traits linked to grain yield and its components. Through this process, heritable changes are introduced, broadening the genetic variability available for selection and thereby strengthening the foundation for genetic enhancement in breeding programs (Mwandaingoni *et al*., 2017). Historically, mutation breeding has been utilized to derive better-performing variants from elite wheat germplasm, contributing to more adaptable and resilient cultivars across various crop species (Shu *et al*., 2012). According to data from the Mutant Variety Database (MVD) and the Food and Agriculture Organization (FAO) of the United Nations, thousands of officially released crop varieties owe their development, either directly or indirectly, to mutation breeding techniques (Mwandaingoni *et al*., 2017). This method relies on the use of physical or chemical mutagens to induce genetic alterations, thereby generating greater diversity. Physical mutagens may include forms of radiation such as corpuscular rays, ultraviolet (UV) light, heat, or electromagnetic radiation, while chemical agents are also commonly used (Mba, 2013). Recent advances have seen these traditional mutagenic techniques integrated with molecular tools, such as the TILLING (Targeting Induced Local Lesions in Genomes) platform, which allows for precise identification of induced genomic changes (Chen *et al*., 2014). The success of this approach depends heavily on the breeder's ability to screen mutant populations effectively and select individuals exhibiting favorable phenotypic traits. These selections then become valuable additions to the genetic pool, offering new opportunities to enhance the adaptability, productivity, and nutritional quality of wheat germplasm (Mwandaingoni *et al*., 2017).

GENETIC ENGINEERING

Biotechnology has enabled the genetic modification of wheat to address the nutritional demands of an expanding global population. Genetic engineering is widely recognized as an effective strategy for increasing the levels of essential micronutrients in wheat, offering a sustainable means of combating dietary deficiencies. Compared to traditional breeding, which often requires several generations of selection, genetic manipulation accelerates the development of new varieties and ensures more stable expression of desired traits (Tewodros, 2015). This approach provides greater precision by allowing the direct insertion of specific genes into the plant's genome, significantly reducing the breeding cycle. Importantly, the inserted genes can originate from a wide array of organisms, including bacteria and animals, expanding the potential for nutritional improvement in crops. In wheat, the objective of genetic engineering is to enhance the concentration of health-promoting compounds, such as inulin, while also reducing anti-nutritional elements and improving the plant's ability to absorb and utilize minerals from the soil (Zhu *et al*., 2007). The process involves linking the target gene to a promoter and marker gene before using a vector to introduce it into the plant's DNA. This results in the development of a transgenic or genetically modified organism (GMO), created through precise genomic integration. Despite its potential, the deployment of genetically engineered crops comes with certain obstacles. One of the major concerns is the association of GMO technologies with intellectual property rights, which can restrict access for public-sector researchers and smallholder farmers (Pardey *et al*., 2000). Nonetheless, the advantages of genetic engineering are substantial, particularly in its ability to regulate a broad spectrum of agronomic and nutritional traits. This makes it a valuable tool in the development of wheat varieties that are better suited to improve human nutrition and support global food security.

STRATEGIES FOR BIOFORTIFICATION

Iron (Fe) and zinc (Zn) are vital for plant development, crop yield, soil health, and nutritional quality. Numerous institutions and research centers have contributed significantly to enhancing the micronutrient content of wheat. Several prominent strategies used for biofortification are outlined below:

1. Fertilizer and Foliar Applications

Micronutrient content in wheat, particularly Fe and Zn, can be increased by applying mineral fertilizers such as zinc sulfate ($ZnSO_4$) and ferrous sulfate ($FeSO_4$). While beneficial, this method is generally not regarded as a long-term sustainable solution (Cakmak, 2008). Among available agronomic approaches, foliar spraying is considered the most effective, as it substantially raises Fe and Zn concentrations in the wheat grain's starchy endosperm (Zhang et al., 2010). More promising results have been achieved when this method is combined with seeds that already possess higher intrinsic Zn levels (Velu *et al*., 2013).

2. Germplasm Evaluation

Extensive screening of over 3,000 wheat germplasm accessions—including diploid, tetraploid, and hexaploid genotypes—has been conducted through the gene bank at CIMMYT to assess Fe and Zn variation (Monasterio and Graham, 2000). Ancestral wheat forms, such as einkorn and wild emmer, as well as landraces, have demonstrated significantly higher micronutrient levels than most modern cultivars (Cakmak *et al*., 2000; Ortiz-Monasterio *et al*., 2007).

3. Genetic Modification (Transgenic Approaches)

Among all current biofortification techniques, transgenic methods are seen as the most cost-efficient and reliable for improving wheat's nutritional profile (Malik et al., 2016). Researchers utilize specific genetic markers to identify loci associated with elevated micronutrient content. A key group of genes known as the ZIP (Zinc-regulated transporter/Iron-regulated transporter-like Protein) family plays a major role in improving Fe and Zn uptake and storage (Schachtman and Barker, 1999; Eide, 2006).

4. Manipulation of Soil Nitrogen and Phosphorus

The presence of phosphorus (P) in soil tends to hinder Fe and Zn absorption due to antagonistic interactions, particularly as about 75% of phosphorus in wheat is stored in the form of phytic acid within the aleurone and germ tissues (Lott and Spitzer, 1980). In contrast, a higher nitrogen (N) status in the plant has been shown to promote root and shoot mobility, translocation of nutrients from vegetative organs to grains, and enhanced partitioning of micronutrients within seeds (Aciksoz *et al*., 2011a; Kutman *et al*., 2010; Erenoglu *et al*., 2011). Increasing nitrogen availability, whether through soil application or foliar feeding, has led to improved uptake and accumulation of Fe and Zn in wheat grains (Kutman *et al*., 2011).

5. Reduction in Glutenin and Plant Height

Plants experiencing Fe and Zn deficiency release phytosiderophores—root-secreted compounds that mobilize these micronutrients for uptake. This process is further enhanced by optimal nitrogen availability (Aciksoz *et al*., 2011b). Research has also shown that grain Fe and Zn levels are inversely related to glutenin content and plant height. Lower glutenin and shorter plant stature are often associated with higher Fe concentrations in the grain (Gomez-Becerra *et al*., 2010a).

6. Use of Plant Growth-Promoting Rhizobacteria (PGPR) and Cyanobacteria

The application of beneficial microbes such as PGPRs—root-colonizing bacteria that stimulate plant growth—represents another promising biofortification strategy. These microbes assist nutrient solubilization and mobilization, and when integrated with genetic improvement programs, they can help enhance wheat's micronutrient content (White and Broadley, 2009).

7. Enhancement of Nicotianamine Levels

Nicotianamine is a crucial chelating compound that supports metal homeostasis in plants by binding to both Fe^{2+} and Fe^{3+} ions depending on soil pH. It helps maintain iron solubility inside cells, improving its availability for cellular processes (Riaz *et al*., 2017). Studies have confirmed its role in enhancing Fe uptake and its deposition in seeds (Douchkov *et al*., 2001; Douchkov *et al*., 2005).

CHALLENGES AHEAD IN BIOFORTIFICATION

The complex interactions among genotype, environmental conditions, grain yield, and nutrient content remain insufficiently understood, in part due to the high cost and labor-intensive nature of phenotyping required for studying nutrient use efficiency. Moreover, evaluating grain quality depends not only on nutrient concentration but also on nutrient bioavailability—an aspect that can be further influenced by climate variability and change. Despite its promise, biofortification faces several obstacles. These include substantial development expenses (Bouis *et al*., 2011; Nestel *et al*., 2006) and uncertainty around the maximum attainable nutrient levels through breeding, particularly when factoring in variables such as adoption by farmers and nutrient losses during food storage and preparation (Taylor and Taylor, 2012).

The process of bringing biofortified varieties to market is lengthy, often taking up to a decade (Bouis *et al*., 2017). This timeline can be extended further for genetically modified crops due to additional political, legal, and regulatory challenges (Birner *et al*., 2007). Another hurdle lies in the limited economic motivation for farmers to cultivate nutrient-enriched crops, along with low consumer awareness regarding their availability and benefits. For biofortification initiatives to succeed, interdisciplinary collaboration is critical. Breeding efforts must ensure that enhanced varieties not only improve nutritional value but also retain desirable traits such as grain appearance, taste, yield potential, and tolerance to both biotic and abiotic stresses.

In addition, more targeted research is needed to evaluate the health impacts of biofortified crops. For example, ongoing studies on iron-enriched varieties are vital for understanding their effectiveness in reducing iron-deficiency anemia and improving broader public health outcomes (Hussain *et al*., 2010).

CONCLUSION

In summary, biofortification presents a promising strategy to enhance the nutritional profile of staple crops like wheat, particularly in addressing widespread deficiencies of essential micronutrients such as iron and zinc. However, several challenges must be overcome to realize its full potential. These include the intricate relationships among genetic factors, environmental conditions, and nutrient accumulation, as well as the high costs associated with research and development. Moreover, ensuring the bioavailability of these nutrients remains a key concern. Political, regulatory, and socioeconomic obstacles including limited farmer incentives and low consumer awareness also pose significant barriers to the adoption of biofortified crops. Achieving success in biofortification requires coordinated, interdisciplinary efforts to develop crop varieties that are not only nutrient-dense but also meet agronomic performance benchmarks and consumer expectations in terms of taste, appearance, and culinary quality. Maintaining productivity and resistance to environmental stresses is equally essential. Ongoing research, innovation, and financial support will be critical for advancing biofortification technologies and integrating them into global food systems, ultimately contributing to the reduction of micronutrient malnutrition and the promotion of public health and food security.

AUTHOR CONTRIBUTION

Author	**Contribution**
Mashal Rehman*	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing

	Has given Final Approval of the version to be published
Usaid Rashid	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Asia Bibi	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Ansaar Ahmed	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Saima Rustom	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Muhammad Usama	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published
Roop Zahra	Contributed to study concept and Data collection Has given Final Approval of the version to be published
Rao Muhammad Tahoor	Writing - Review & Editing, Assistance with Data Curation
Rima Bibi	Writing - Review & Editing, Assistance with Data Curation
Faria Pervaiz	Writing - Review & Editing, Assistance with Data Curation

REFERENCES

1. Aciksoz, S.B., Yazici, A., Ozturk, L., Cakmak, I. (2011a). Biofortification of wheat with iron through soil and foliar application of nitrogen and iron fertilizer. Plant soil, 215-349.
2. Aciksoz, S.B., Ozturk, I., Gokmen, O.O., Roemheld, V., Cakmak, I. (2011b). Effect of nitrogen on root release of phytosiderophores and root uptake of Fe (III) - Phytosiderophore in Fe-deficient wheat plants. Physiol. plant, 142, 287-296.
3. Ahmadi J., Pour- Aboughadareh A., Ourang S.F., Mehrabi A., Sidduqe K H M. (2018) Wild Relatives of Wheat: Ageliops Triticum accessions disclose differential anti-oxidative and physiological responses toward water stresses. Asta Physiological Plant, 40, 1-14
4. B.J Alloway (2009). Soil Factors associated with zinc deficiency in crops and humans. Environment Geo Chemistry Health, 31, 537-548
5. Belderok, B. (2000). Bread making quality of wheat: A century of Breeding in Europe. Kulwer Academic Publisher Belderok, Netherlands, Pp;34
6. Birner, R., Kone, S.A, Linarce,N., and Resnick,D (2007). Biofortified food and crops in west Africa; Mali and Burkina Faso AgBioForum 10(3) : 192
7. Bouis H.E., Saltzman, A (2017). Improving nutrition through Biofortication: A Review of evidences from Harvest Plus., 2003 through 2016. Global Food Security, 12, 49-58.
8. Bouis.H.E., Hotz.C., MC. Clafferty B., Meenakshi, J.V & Pfeiffer., W.H (2011). Biofortication: A new tool to reduce micronutrient malnutrition. Food Nutrition Bull; 32: 31-40.

9. Breseghello F., Coelho A.S.G (2013). Traditional and Modern Plant Breeding Methods with Examples in Rice (Oryza sativa L)., J Agric Food Chemistry., 61,8277-8286
10. Cakmak, Ismail. (2008) Enrichment of cereal grains with zinc: Agronomic or genetic biofortification? Plant Soil, 302, 1–17.
11. Cakmak I., Pleiffer W.H & Mc Clafferty B (2010a) Review; Biofortication of durum wheat with zinc and iron. AACC International Inc., Cereal Chemistry 87(1), 10-20.
12. Cakmak I., Torum A., Millet E., Feldman M., Fahima T., Korol A., Nevo E., Braun H.J., Ozkan H., (2004). Triticum Dicoccoides: An important genetic resource for increasing zinc and iron concentration in modern wheat. Soil Science, Plant Nutrition. 50, 1047-1054
13. Cakmak L., Kalaya M., Kaya Y., Torun AA., Aydin N., Wang Y., Arisoy Z., Erdem H., Yazici A., & Gokmen O. (2010). Biofortication & Localization of Zinc in Wheat Grain. Jagric Food Chain. 58, 9092-9102. DOI: 10.1021/jf101197h.
14. Cakmak, I., Ozkan, H., Braun, H.J., Welch, R.M., Romheleld. (2000). Zinc and iron concentrations in seeds of wild, primitive and modern wheat. Food Nutr. Bull, 21, 401-403.
15. Chen L., L Hao., M.A.Parry., A.L.Phillips & Y.G.Hu (2014) Progress in tilling as a tool for functional genomics and improvement of crops., Journal of integrative plant Biology 56: 425-443
16. Chhuneja P., Kaur S., Goeal R.K., Aghaee M., Sarbarreb M., Prasbar & H S Dhaliwal (2008). Transfer of leaf rust and stripe rust resistance from Aegliops Umbelluta Zhuk to Bread Wheat (*Triticum aestivum*). Genetic Resources Crop Evolution, 55, 849
17. Cui Y., Zhong F., Xu J., Li Z., Xu S., (2015) Mapping quantitative trait loci in selected breeding populations: A Segregation distortion approach., 115, 538-546
18. De Valenca A W., Bake A., Brouwe l.D., Giller K.E. (2017) Agronomic Biofortication of crops to fight hidden hunger in sub-saharan Africa. Global Food Security,12, 8-14.
19. Debasis, P., and Khrrana, P. (2001). Wheat Biotechnology: A mini-review. Electronic J, Biotech., 4(2), 74-102, DOI: 10.4067/S0717-34582001000200007 Dempewolf H., Baute G., Anderson J., Killian B., Smith C., Guarion L., (2017). Past and future uses of wild Relative in Crop Breeding. Crop science, 57, 1070-1082.
20. Douchkov D., Hell R., Stephan UW, and Baumle in H.(2001). Increased iron efficiency in transgenic plants due to ectopic expression of nicotianamine synthase. Plant Nutr. 92:54-55.
21. Douchkov D., Gryczka C., Stephan U.W., Hell R., and Baumlein H.(2005). Ectopic expression of nicotianamine synthetase genes results in improved iron accumulation and increased nickel tolerance in transgenic tobacco plant cell. Envir.28: 365-374.
22. Fageria Nk. & Baligar VC. (2008) Ameliorating soil acidity of tropical oxisols by liming for crop production. Adv Agron, 99: 345-399. DOI: 0.1016/S0065-2113(08)00407-0
23. Fageria Nk, Baligar VC, Clark R B (2002). Micronutrients in crop production. Adv Agron 77, 85-268. DOI: https://doi.org/10.1016/S0065-2113(02)77015-6
24. Frossad E., Bucher M., Machler F., Mozafar A., Hurrell R., (2000). Potential for increasing the content and bioavailability of Fe, Zn & Ca in plants for human nutrition. J.Sci Food Agric, 80, 861-879. DOI: https://doi.org/10.1002/(SICI)1097.
25. G. Velu, I.Ortiz-Monasterio, I.cakmak, Y.Hao, R.P Singh (2013). Biofortication strategies to increase grain Zinc& Iron concentration in Wheat. Journal of cereal science, 59(3), 365-
26. Garg M., Sharma N., Sharma S., Kapoor P., Kumar A., Chandari V., Arora P. (2018). Biofortified Crops generated by Breeding agronomy & Transgenic Approaches are improving lives of millions of people around the world. Front Nutr, 5, 12.
27. Gomez- Becerra, H.F. Erdem., H. Yazici., A.Tutus, Y., Torun B., Ozturk L., Cakmak I.(2010 b). Grain concentration of protein and mineral nutrients in a large collection of spelt wheat grown under different environments. Journal Cereal Science, 52(3), 342-349.

28. Gomez-Becerra, H.F. Yazici, A., Ozturk, L., Budak, H., Peleg, Z., Morgounov, A., Fahima, T., Saranga, Y., Cakmak, I. (2010a). Genetic variation and environmental stability of grain mineral nutrient concentrations in Triticum dicoccoides under five environments. Euphytica, 171, 39-52.

29. Gomez-Galera S., Rojas E., Sudhakar D., Zhu C.F., Pelacho A.M., Capell T., etal (2010) Critical evaluation of strategies for mineral fortification of staple food crops., Transgenic Resources 19 165-180.

30. Grew,R (2018). Food in global History. Routledge, NewYork, NY USA.

31. Grusak, M. (2002). Enhancing mineral content in plant food products. J.Am Cell Nutrition, 21(3), 1785-1835.

32. Gupta P.K., Kumar J., Mir RR& Kumar A. (2010). Marker- Assisted selection as a component of Conventional plant breeding in wheat, 33(145).

33. Hussain S., Maqsood A M & Rahmatullah (2010). Increasing grain zinc and yield of wheat for the developing world: A Review. Emir. J. Food Agric, 22 (5): 326-339.

34. Kutman, U.B., Yildiz, B., Cakmak, I. (2011). Effect of nitrogen on uptake, remobilisation and partitioning of zinc and iron throughout the development of durum wheat. Plant soil, 342, 149-164.

35. Li M., Wang S., Tian X., Li S., Chen Y., Jia Z., Liu K., Zhao. A. (2016). Zinc and iron concentration in grain milling fractions through combined foliar application of Zn and macronutrients. Field Crops Res, 187, 135-141

36. Lott, J.N.A., Spitzer, E. (1980). X-ray analysis studies of elements stored in protein body globid crystals of triticum grains. Plant physiology, 66(3), 494-499.

37. Ludwig Y. and Slamet-Wedin I.H., (2019). Genetic Biofortication to enrich rice and wheat grain Iron: From Genes to Product.Frontiersin Plant Science. 10, 833.

38. Mara, C & Petra. B. (2012). Strategies for Iron Biofortication of Crop Plants. Food quality source in Technology, 2, 953-978.

39. Mba C, (2013). Induced mutations unleash the potential of plant gentic resources for food and agriculture. Agronomy Journal, 3(1), 200-231.

40. Mohammadi-joo S., Mirasi A., Saediaboeshaghi R., and Amiri M., (2015) Evaluation of bread wheat (Triticum aestivum L.)genotypes based on resistance indices under field conditions.Intl I Biosci 6 (2): 331-337.

41. Monasterio I. and Gresham R.D.(2000)Breeding for trace minerals in wheat, Food nutrition Bull, 21(4), 393-396. DOI: https://doi.org/10.1177/156482650002100409

42. Moose S.P., Mumm R.H. 2008. Molecular plant breeding as the foundation for 21st century crop improvement. 147, 969- 977.

43. Morgounov, A., Gomez-Becerra, H.F., Abugalieva. A., Dzhunusova M., Yessimbekova M., Muminjanous H., Zelenskiy Y., Ozturk I., Cakamak I., (2007) Iron and zinc grain density in common wheat grown in central Asia. Europhytica 155, 193-103.

44. Mulualem, T. 2015.Application of biofortication through Plant breeding to improve the value of staple crops. Blomed Biotechnol, 3,11-19

45. Mwandaingoni L., Figlan S., Shimelis H., Mondal S., & Tsilo. T.J. (2017). Genetic resources and breeding methodologies for improving drought tolerance in wheat. Journal of crop improvement, 31(5), 648-672.

46. Nestel,P., Boius, J.V., Meenakshi, J.V, and Pfeiffer,W. (2006) Biofortication of stable food crops. J.Nutr., 136(4): 1064-1067. DOI: DOI: 10.1093/jn/136.4.1064

47. Ortiz-Monasterio I., Palacios-Rojas N., Meng E., Pixley K., Trethowan R., Pena R.J., (2007). Enhancing the mineral and vitamin content of wheat and maize through plant breeding. Journal of Cereal Science, 46(3), 293-307.

48. Pardey P G., Wright B D., & Nottenburg C., (2000). Are intellectual property rights shifting agricultural bio-technology in developing countries? International Food Policy Research Institute (IFPRI), Washington DC.

49. Peleg Z., Aranga Y., Yazici A., Fahima T., Oztruk L., Cakmak L., (2007). Grain zinc iron & protein concentration & Zinc efficiency in wild emmer wheat under contrasting irrigation regimes. Plant soil. 306(1-2), 57-67.

50. Pleiffer, W.H., & M C Claffererty, B (2007) Biofortification: Breeding micronutrient dense crops,Breeding major food staples M.S.kang & P.M Priyadarshan. Eds Blackwell publishing Oxford, 61-9

51. Qaim M., Stein AJ.& Meenakshi J V. (2007)Economics of Biofortication. Agr Economics-Blackwell B7 (s1), 119-133. DOI: 10.1111/j.1574-0862.2007.00239.x

52. Rawat N., V.K. Tiwari, P. Chhuneja and H.S Phaliwal, (2009). Evolution and utilization of Aegilops & Wild Triticum species for enhancing iron and zinc content in wheat. Genetic Resources Crop Evolution. 56(1), 53-64.

53. Rengel Z, Batten GD, Crowley DE (1999). Agronomic approaches for improving the micronutrient density in edible portion of field crop. Felid crop Res 60, 27-40.

54. Riaz A., Huda NL. Abbas A., Raza S (2017). Biofortification of wheat with iron. International journal of advances in Scientific Research; 3(07): 69-76.

55. Ruel M.T., Alderman.H. (2013). Nutrition-sensitive interventions and Programmes: How can they help to accelerate progress in improving material and child nutrition? Maternal and child nutrition study group., Lancet., 382(2013), 536-551

56. Saini D.K., Devi.P., and Kaushik.P. Advances in genomic interventions for wheat biofortification. A review (2020); Agronomy 2020.10,62.

57. Shao Y., Jin L., Zhang G., Lu,Y; Shen,Y, Bao J. (2011) Association mapping of grain colour, phenolic content flavonoid content and antioxidant capacity in deshelled rice.Theoryand Applied Genetics, 122(5), 1005-1016. DOI: 10.1007/s00122-010-1505-4

58. Sharma S., Chunduri V., Kumar A., Kumar R., Khare P., Kondepudi K.K., Bishnoi M., Garg M., (2018) Anthocyanin Bio-fortified coloured wheat : nutritional and functional characterization, Plos One, 13, e0194367. DOI: https://doi.org/10.1371/journal.pone.0194367

59. Shu Q., B.P.Forster., H.Nakagawa, & H.Nakagawa (2012). Plant mutation breeding and biotechnology,FAO, CABI. http://www.fao.org/3/a-i2388e.pdf

60. Singh.U., Prahara, C.S.,Chaturvedi, S.K., and Bohra, A. (2016) Biofortication: Introduction, Approaches, limitation and challenges. Springer India; DOI: https://doi.org/10.1007/978-81-322-2716-8_1

61. Statistica (2020). Global wheat production from 2011/2012 to 2019/2020 (in million metric tons). https://www.statista.com/statistics/267268/production-of- wheat-worldwide-since-1990/

62. Taylor, J, and Taylor, J.R.N, (2012) Cereal Biofortication: Strategies, Challenges, and Benefit, Cereal Food World, 57, 165-169. https://www.cerealsgrains.org/publications/plexus/cfw/pastissu es/2012/Documents/CFW-57-4-0165.pdf

63. Tewodros Mululemi (2015). Application of Bio-fortification through plant breeding to improve the value of staple crops. Biomedical and Biotechnology, 3(1), 11-19. DOI: 10.12691/bb-3-1-3

64. Trethowan R.M., Reynolds M.P., Ortiz-Monasterior., (2005). Adapting wheat cultivars to resource conserving farming practices and human nutritional needs. An Applied Biology, 146,404-419. DOI: https://doi.org/10.1111/j.1744 7348.2005.040137.x

65. Trethowan R.M., Reynolds M.P., Ortiz-Monasterior., Ortiz R. (2007). The genetic basis of green revolution in wheat production. Plant Breed REV-28, 39-58. DOI: https://doi.org/10.1002/9780470168028.ch2

66. Tulchincky, T.H. (2010) Micronutrient Deficiency Conditions: Global Health Issues. Public Health Rev 32, 243–255. DOI: https://doi.org/10.1007/BF03391600

67. Welch R.M., Graham R.D (2004) Breeding for Micronutrients in staple food crops from a human nutrition perspective. J.Exp Bot. 55 353-364. DOI: 10.1093/jxb/erh064

68. White P.J & Broadely M.R. 2009. Biofortication of crops with seven mineral elements often lacking in human diets-iron, zinc, copper, calcium, magnesium, selenium & iodine. New Phytologist, 182, 49-84. DOI: https://doi.org/10.1111/j.1469-8137.2008.02738.x

69. Zhu C., Naqvi S., Gomez-Galera S., Pelacho A.M., Capell T., Christon P (2007). Transgenic strategies for the nutritional enhancement of plants. Trends Plant Science, 12(12), 548-565.DOI: https://doi.org/10.1016/j.tplants.2007.09.007

