



Systematic Review

"Bio-Fortification for Wheat: Enhancing Zinc and Iron Nutritional Quality to Combat Micro-Nutrient Deficiencies"

Muhammad Adeel Ahmad¹, Hafiz Muhammad Jahanzab², Sibghatullah³, Muhammad Nouman Malik⁴, Muhammad Ismail Malik⁴, Malik Ali Nawaz⁴

1 Faculty of Agriculture Department of Soil science, Gomal University Dera Ismail Khan, Pakistan.

2 Faculty of Agriculture Department of Agronomy, University of Agriculture Dera Ismail Khan, Pakistan.

3 Agriculture Research Center D.I. Khan, Pakistan.

4 Faculty of Agriculture Department of Agronomy, Gomal University Dera Ismail Khan, Pakistan.

ARTICLE INFO

Received: 28 July 2025

Revised: 30 August 2025

Accepted: 15 September 2025

Published: 31 December 2025

Key Words:

*Wheat Biofortification
 *Zinc
 *Iron
 *Micronutrient Deficiency
 *Crispr
 *Genetic Engineering
 *Harvest plus
 *Nutritional Security

*Corresponding Author:

Muhammad Adeel Ahmad
adeelahmad7773@gmail.com

ABSTRACT

wheat (*Triticum aestivum* L.), a staple grain that contributes to global food security. However, common micro-nutrient shortages, especially in zinc (Zn) and iron (Fe), are frequently not addressed by its function as a staple. Nearly 2 billion individuals experience hidden hunger, which shows up as stunted growth, anemia, and weakened immunity system. With an emphasis on genetic, agronomic, and biotechnological methods, this review critically assesses developments in bio-fortification techniques to raise the Zn and Fe content of wheat grains. Wheat is a staple crop for billions, provides insufficient levels of bioavailable Fe and Zn, contributing to “hidden hunger” in developing regions. Soil conditions such as high pH further limit Fe and Zn uptake in wheat, Soil application of zinc and iron or foliar application of zinc and iron should adopt in wheat cropping to Improve bio-fortification. Bio-fortification through Foliar spray, Soil application breeding based, approaches has emerged as a sustainable solution. all of which have identified key locally and functional genes regulating Fe and Zn uptake, transport, and storage. wheat bio-fortification is a cost-effective, scalable strategy to combat micronutrient malnutrition and promote food security. Multidisciplinary researchers working on wheat bio-fortification may find this article to be a useful resource, helping to improve present and future initiatives to achieve the Sustainable Development Goal of eradicating hunger.

INTRODUCTION

Micronutrient deficiencies especially of zinc and iron are a widespread world health issue, impacting billions of people and causing serious health issues, such as weakened immunity, retarded development, and anemia (Sharma et al., 2020). Biofortification of staple crops such as wheat (which contributes about 20% of all global calories and protein in the diet) has the potential to be a viable and affordable solution to this hidden hunger (Devate et al., 2023; Sheera et al., 2025). It is concerned with the principle of raising the natural level of iron and zinc in the grains of wheat and, therefore, will be a more sustainable and less expensive source of nutrition compared to the traditional techniques of fortification (Borrill et al., 2014; Chakraborty and Shrivastav, 2024). Being one of the key food resources of the population in the majority of developing countries, the improvement of the nutritional value of cereals, in particular, the required micronutrients that are frequently lost during processing, is a burning issue (Sharma et al., 2020). This review will assess the progress of biofortification of wheat, particularly the genetic, agronomic and biotechnological mechanisms of fortifying the wheat with zinc and iron (Sheera et al., 2025). It is worth mentioning that half of all soils that cereals

grow on the Earth lack zinc, and the lack of iron dominates in arid and calcareous areas, which underlines the urgent necessity to biofortify to be able to guarantee food security (Wani et al., 2022). One such practice is a genetic biofortification practice, which involves using the genetic diversity that is already in place in wheat and its wild counterparts to develop varieties that will be rich in nutrients that will be sustained at the micronutrient level thus offering a permanent solution to this common problem (Nasim et al., 2025; Wanyera and Owuoché, 2017). This biological approach avoids the problems of traditional breeding, including the need to apply time-consuming phenotyping, in favor of cutting-edge genomic technology, including Quantitative Trait Loci mapping and Genome-Wide Association Studies to discover desirable genetic loci (Chakraborty and Shrivastav, 2024). These genomic methods would make it possible to determine genetic markers associated with excessive amounts of zinc and iron that would be used to select a marker-based breeding program (Kumar and Kaushik, 2023). Moreover, it can be targeted to adjust the regulatory pathways overseeing mineral absorption, translocation, and sequestration in the grain with such genomic understanding to maximize the bioavailability of essential micronutrients

(Sun et al., 2023). This is particularly relevant because nutritional value of commercialized versions of wheat has been on the decline over the years due to the focus on productivity and disease resistance (Nasim et al., 2025). The deficiency of micronutrients can be seen in the world population, which is why the interest in such biofortification actions is urgent, with over three billion people iron and zinc-deficient, with the majority being in the developing countries that use cereals (Sharma et al., 2021). As one of the staples of almost one-third of the world population, wheat is a major target of intervention, especially as its natural bioavailability of zinc and iron is usually low, especially with growth in zinc-depleted soils (Sharma et al., 2021). This is further enhanced by the fact that fertilizers that contain minimal amounts of minerals are still being used and do not replenish the soil of essential micro-nutrients that exacerbate the inability to obtain wheat crops (Sharma et al., 2020). Therefore, bio enrichment of wheat varieties through enhancement of their inherent levels of micronutrients is an emergency effort to decrease malnutrition in the world (Ali and Borrill, 2020; Krishnappa et al., 2022). In particular, genetic biofortification of wheat is geared to raise the level of iron and zinc in the grain to 59 ppm

and 38 ppm respectively to achieve 30% and 40% of the recommended average intakes of the two nutrients in children and adult women respectively, beginning with a 30-ppm level of iron and 24 ppm of zinc in the grain. An example such as genomic selection is more comprehensive than marker-assisted selection since it predicts genetic value using higher-thruster genomic structures of markers, thus, a larger share of genetic variation in characteristics such as micronutrient content (Gupta et al., 2024). This system thinking can greatly hasten the creation of biofortified wheat strains with improved nutritional worth, which will tackle the micronutrient malnutrition issue afflicting the planet (Nasim et al., 2025; Sharma et al., 2021). The key to the rapid production of the high-quality biofortified forms of wheat, therefore, lies in the tactical use of the high level of breeding techniques and molecular instruments like the identification of a single nucleotide polymorphism that is linked to the abundant content of micronutrients (Krishnappa et al., 2022). This is necessary, as micronutrient malnutrition, zinc and iron in particular, affects over 700 million people on a global scale and biofortification proves to be a cost-effective and sustainable solution to the problem of this common health concern (Sun

et al., 2023). As an alternative to fortification, another method of micronutrient delivery into processed food, biofortification implies increased nutrient levels in the crop itself, and is a more feasible and sustainable intervention to protect at-risk groups in remote or underserved areas (Gupta et al., 2024). Over the last few years, the biofortification of wheat has now been greatly contributed to by genomic interventions including quantitative trait loci mapping, marker-assisted selection, and genomic selection (Saini et al., 2020). Such molecular methods along with the genome-wide association studies play a significant role in the deciphering of the complex genomic basis of micronutrient accumulation in wheat and the discovery of novel genomic areas with heightened grain mineral contents (Gupta et al., 2022; Krishnappa et al., 2021). In another case, multi-trait genomic prediction models have been demonstrated to be more precise in predicting traits of selenium and iron than single-trait models that result in increased breeding efficiency (Tadesse et al., 2023). In addition, emerging technologies that could identify causal variants and provide a detailed perspective of micronutrient deposition at both cellular and molecular levels include the use of genome editing and integrating functionome data into

GWAS analyses (Gupta et al., 2022; Kumar et al., 2025). These new genetic and genomic technologies cannot be ignored in the endeavor to come up with new types of wheat with a superior micronutrient status, which is a critical move towards combating micronutrient deficiency in the world (Ali and Borrill, 2020; C et al., 2022; Ibba et al., 2022). This approach is particularly significant in those regions where the main source of food is cereals and the dietary diversification is low and biofortified varieties are not available, which is a source of hidden hunger (Bhattacharya et al., 2024; Manjunath et al., 2023). In addition, the development of CRISPR/Cas-based genome editing presents some editing possibilities on the genes that control the storage of nutrients that can be leveraged in conjunction with the conventional breeding and genomic selection methods (Kumar et al., 2025). The changes in the gene expression pathways which control the micronutrient transporters and storage proteins can be targeted by CRISPR/Cas technology to allow a more effective uptake and storage of zinc and iron in the wheat grain (Bhattacharya et al., 2024; Kumar et al., 2025). The genetic engineering method along with the other biotechnological methods such as the utilization of natural genetic variation of wheat and its wild relatives using QTL

mapping and genome-wide association analysis enable the identification and use of desirable alleles to improve micronutrient content (Gupta et al., 2022; Sen et al., 2024). The transgenic strategy, i.e., CRISPR / Cas9, is a fast method of enriching the level of iron and zinc in the wheat grains, i.e., in the endosperm, and assists in comprehending the functions of candidate genes (Bhattacharya et al., 2024; Kumar and Kaushik, 2023).

Global Burden of Micronutrient Deficiencies and the Role of Wheat

Micronutrient deficiencies are a global health challenge with extensive health, economic and social justice implications. Globally, about 17 percent of the population is affected by zinc deficiency, and more than 30 percent al., 2021).

of the population in the areas where wheat forms the major staple food is affected, such as South Asia, parts of Sub-Saharan Africa, and the Middle East (Kumssa et al., 2015). Iron deficiency anemia is considered to be one of the most common nutritional diseases in the world and is estimated that there are 1.62 billion people with this disease today; women and children are over represented in developing countries. The health consequences of these deficiencies cut across the lifecycle, where children have reduced physical growth and cognitive growth, mothers are more likely to die, work productivity is lower, and the immune functions of adults are compromised (Siyal et

Region	Population (millions)	Wheat as Staple (%)	Zinc Deficiency (%)	Iron Deficiency (%)	At-Risk Population (millions)
South Asia	1,850	35	28	52	520
Sub-Saharan Africa	1,140	15	24	45	274
Middle East & North Africa	580	65	19	38	224
Central Asia	180	75	22	41	83
Latin America & Caribbean	650	25	16	28	165
Total	4,400	Variable	22 (avg)	40 (avg)	1,266

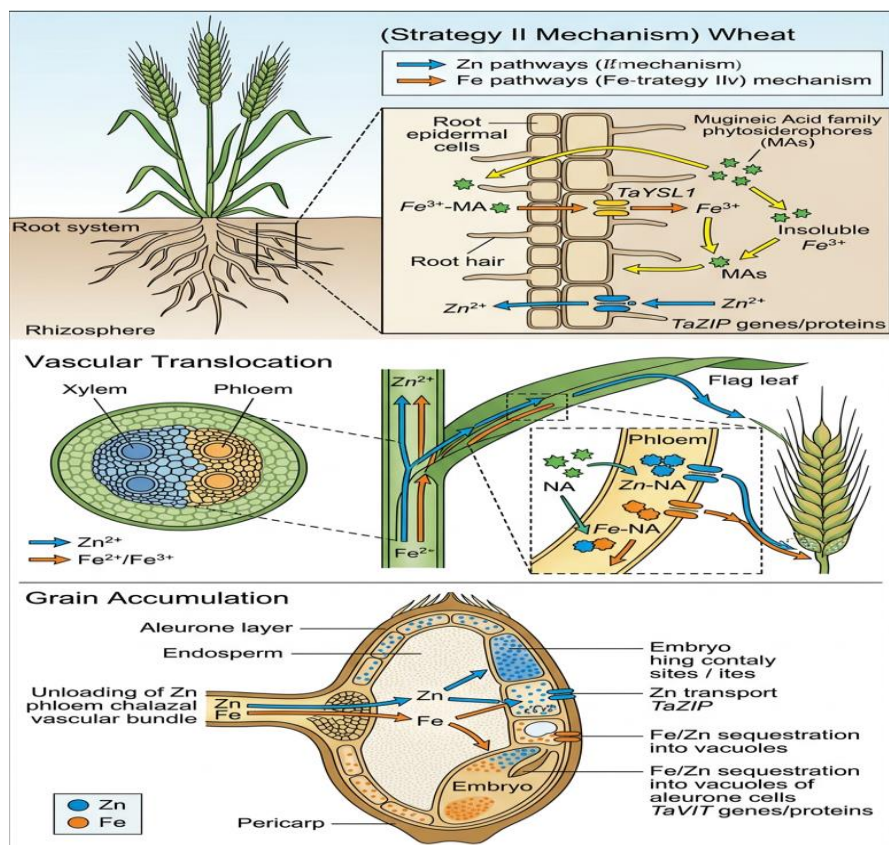
Source: Adapted from Kumssa et al. (2015) and Harvest Plus (2024)

The pathophysiology of the health impacts of zinc and iron deficiencies is connected in a variety of different ways. Zinc is a co-factor in over 300 enzymes and it has a wide range of effects on physiological processes (protein synthesis, DNA synthesis, cell division and immune response). Deficiency is a growth retardation, decreases infection resistance and cognitive development (Hess and Brown, 2017). Iron deficiency greatly affects the oxygen transportation and cellular respiration due to the deficiency of hemoglobin production leading to anemia and subsequent fatigue, work ability and cognitive dysfunction. Severe iron deficiency during pregnancy has been documented to risk the maternal mortality and preterm births and low birth weights.

Food systems based on wheat are especially prone to micronutrient deficiencies because of a number of reasons. First, there is a bias to the modern types of wheat, that have been chosen mainly on the basis of yield, resistance to diseases and processing quality which dilute the level of micronutrients unintentionally. Second, milling and refining process to produce white flour destroys the

layers of bran and germ rich in nutrients and minerals and removes zinc and iron by 60-80. Third, the phytic acid in the bran layer of wheat chelates minerals and decreases their bioavailability and the bioavailability of minerals in normal diets containing whole grain wheat is only 15-25% (Singh et al., 2022).

Wheat-based populations are still economically high in terms of the cost of their micronutrient deficiencies. The Indian and Pakistani literature shows that the cost alone of the zinc deficiency deprives these two nations billions of dollars of productivity and health care expenditures in a year. Interventions provided through bio fortification programs are highly cost effective and the ratio of benefit-cost is more than 20:1 in high prevalence areas. The possible effect is both on the immediate health gains and on enhanced educational achievement, enhanced economic output and decreased healthcare systems burdens and has multiplier effects across the society.



Mechanisms of Zinc and Iron Uptake and Accumulation in Wheat Grains

The biofortification strategies are founded on the basic understanding of the physiological processes that control the uptake, translocation and storage of zinc and iron in wheat grains. The two micronutrients have complex pathways of acquiring their soils, their intake by the roots, their transportation through the vascular, their remobilization of the vegetative tissues and their eventual deposits in growing grains (Figure 3).

Strategy II iron uptake mechanisms are used by graminaceous plant species like wheat, and have analogous chelation-based iron uptake mechanisms. Strategy II: mugineic acid family phytosiderophores (MAs)

synthesis and release are regulated by strategy II: root cells and chelate sparingly soluble Fe (III) and Zn (II) in the rhizosphere. The resulting Fe (III)-MA and Zn (II)-MA complexes are then taken into root cells via some yellow stripe (YS) transporters, primarily TaYS1 in wheat. The technique also comes in handy in alkaline soils where the potential of the normal uptake processes that take place are constrained by the minute quantities of solubility of the micronutrients. Once absorbed into roots, zinc and iron are transported by complex translocation routes which involve a number of transport processes. Nicotianamine is a non-

proteinogenic amino acid produced as a result of the condensation of three molecules of S-adenosylmethionine by nicotianamine synthase (NAS) and binds both Zn (II) and Fe (II) forming stable complexes and is the main chelator in phloem transport. Transporters of the Yellow Stripe-Like (YSL) family transport these metal-nicotianamine complexes into growing grains, into the endosperm, and into aleurone (Beasley et al., 2022).

There are also several quantitative trait loci (QTL) of genetic studies that are linked to grain levels of zinc and iron and they are a marker-assisted selection object in breeding programs. Velu et al. (2018) conducted genome-wide CIMMYT wheat germplasm genome-wide association studies that revealed SNP significant association on 2A, 2D, 4A, 5A, 5B, 6B, and 7A chromosomes that were correlated with the grain zinc concentration. All these QTL accounted 15-25% of the variance in phenotype which is indicative of polygenic control with medium heritability. Analyses of iron concentration identified similar QTL on chromosomes 2A, 4A, 5B, 6A and 7B, some of which overlap with zinc QTL, suggesting pleiotropy or an interacting gene.

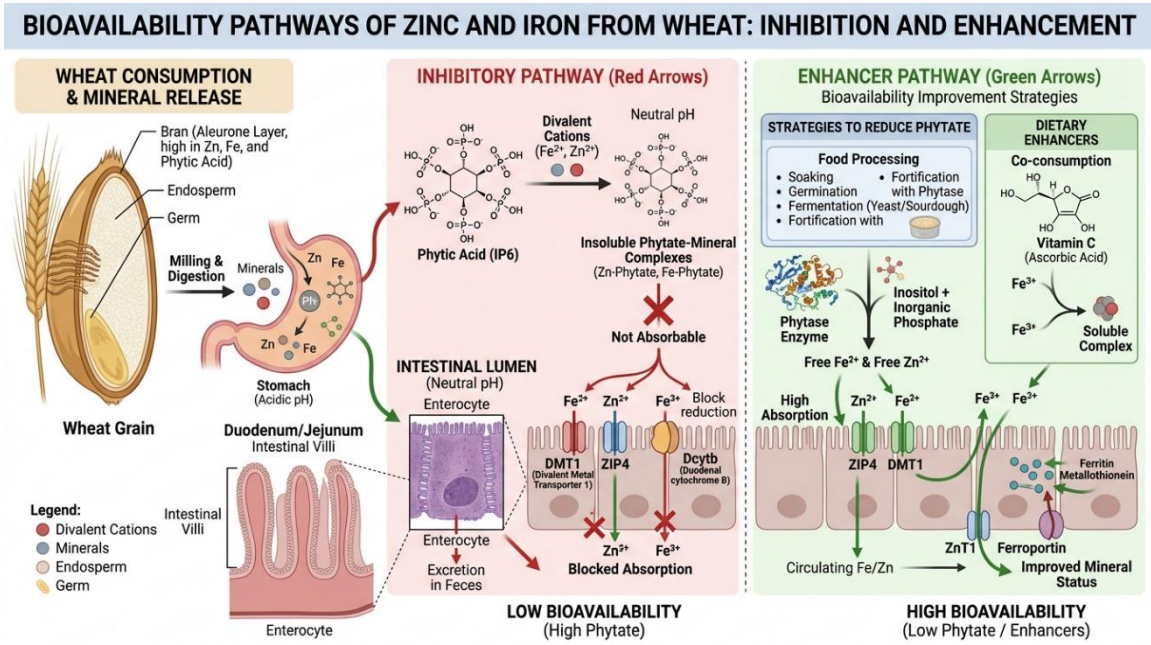
Gpc-B1 gene, which is a NAC transcription factor that was initially discovered in wild

emmer wheat (*Triticum turgidum* ssp. *dicoccoides*), is one of the most important findings in the biofortification of wheat. A study done by Uauy et al. (2006) established that the functional Gpc-B1 allele hastens senescence, and improves the mobilization of nutrients to vegetative tissues to growing grains, which boosts grain protein, zinc and iron levels by 10-15 percent without reducing yields. One of the primary breeding goals is the efficiency of remobilization of nitrogen and micronutrients that is influenced by this gene.

Another important process that is significant and defines the micronutrient content of grain is the vacuolar sequestration. Iron loading into vacuoles during the development of grains is through the vacuolar iron transporter (VIT) family and TaVIT2 in wheat. The process supplies the iron storage in the cells, but may deplete endosomal bioavailable iron. The recent studies have focused on VIT genes in order to add more iron in the endosperm fraction; the bulk of the flour that is used in refined products. Beasley et al. (2022) demonstrated that the expression of endosperm-specific VIT2 in the expression of NAS dramatically increased the level of iron and zinc in white flour, overcoming an important limitation of conventional breeding.

The buildup of nutrients in the grains of wheat could also be related to the stage of development which might provide a good understanding of the bio fortification practices. Zinc and iron build-up further occur during grain filling phase and an estimated 70-80 percent of end grain micronutrient content deposition occurs during 10-25 days post anthesis. Direct root uptake during grain filling, and remobilization of vegetative tissues, particularly flag leaves and stems, lead to the accumulation. The proportion of the sources also depends on genotype, environmental factors and nutrient concentrations, and usually remobilization contributes 40-60 percent of the ultimate grain content in the field.

Such physiological mechanisms have been known to allow some interventions in other biofortification processes. The natural difference in uptake, translocation and remobilization efficiency can be exploited in traditional breeding. Specific pathway components can be improved using genetic engineering to increase NAS expression to be better at chelation and transport. The times of uptake are important and Agronomic practices would be employed to assure that the availability of the soil and the time of the application of fertilizer are in contact with the uptake time. Cas9/CRISPR editing can be used to edit single genes that control vacuolar sequestration or anti-nutrient synthesis to enhance bioavailable mineral content in consumed grain fractions.



Conventional Breeding for Biofortification

The most common and accessible to farmers type of wheat biofortification is conventional breeding where natural genetic variability is used to formulate better varieties through systematic breeding and selection. Starting in the early 2000s, Harvest Plus-CIMMYT had begun screening accessions of wheat germplasm in large-scale efforts, screening thousands of accessions in the CIMMYT genebank and national collections to identify sources of high grain zinc and iron levels (Velu et al., 2018).

In the initial screening, it was found that there was a high degree of genetic diversity in the zinc content of the grain with a range of 15 to 55 mg/kg amongst the cultivated varieties of wheat and up to 90mg/kg in the wild varieties. This disparity is what breeding programs had to achieve the level of 38mg/kg of zinc and 59mg/kg of iron which is the goal of the Harvest Plus. But initial failure was encountered because of poor agronomic behavior of the high nutrient lines such as low

yield, low disease resistance or low processing qualities and the necessity to undergo many backcrossing with the elite lines.

Breeding progress was speeded up by the development of molecular markers associated with high zinc and iron QTL. KASP markers of important QTL at chromosomes 2A and 7A were designed by Gupta et al. (2022), which is why it was effective to conduct a marker-assisted selection at an earlier generation. These markers not only shortened breeding cycle to 2-3 years but also enhanced the precision of choice particularly when there is moderate trait heritability as well as a high degree of interaction between genotype and the environment. Single genetic backgrounds resulted in several QTL, which had a combined effect on the concentration of grain micronutrients, with marker-assisted recurrent selection plans.

Variety Name	Country	Year	Grain Zn (mg/kg)	Grain Fe (mg/kg)	Yield Penalty (%)	Area (000 ha, 2023)
Zincol-2016	Pakistan	2016	38.2	48.5	0	245
BARI Gom 33	Bangladesh	2017	40.1	52.3	-2	180
Zinc Shakti	India	2018	39.5	50.2	-1	320
Akbar-19	Afghanistan	2019	37.8	47.6	0	45
HB-2015	Pakistan	2015	36.9	46.8	0	165
WB2	India	2015	38.7	51.4	-3	210

NARC-2017	Pakistan	2017	37.4	49.1	0	142
PAK/15/0	Pakistan	2019	38.9	48.3	0	118
PBW-Golden	India	2020	41.2	53.6	-2	95
Atta Habib	Afghanistan	2020	36.5	47.2	0	38
Etiopía Zinc	Ethiopia	2021	39.8	48.9	-1	52
Zinc Sonora	Mexico	2016	37.2	46.5	0	78

***Source:** Harvest Plus (2024) and Kumar et al. (2022) *

These varieties achieved the desired level of concentration of zinc in Harvest Plus and agronomical performance comparable to the conventional check varieties. Curiously, the vast majority of releases were not harshly or overly punished in yield and this is an important issue that once restrained the use of nutritionally enhanced varieties. Multi-location testing of target environment showed consistent expression of high levels of micronutrients with genotype environment interaction which explained 15-25 percent of the variation in grain zinc level.

This breeding approach that was followed by Harvest Plus and CIMMYT was systematic introgression of high zinc alleles into elite, locally adapted backgrounds through use of backcross breeding and the application of a marker assisted selection. Backcross 2-3 generations were usually sufficient to recapture 95-98 percent of recurrent parent genome with a high level of zinc alleles. This plan allowed the biofortified varieties to

retain the disease resistance, agronomic performance and final use quality attributes to be established by the farmers and taken up by the market.

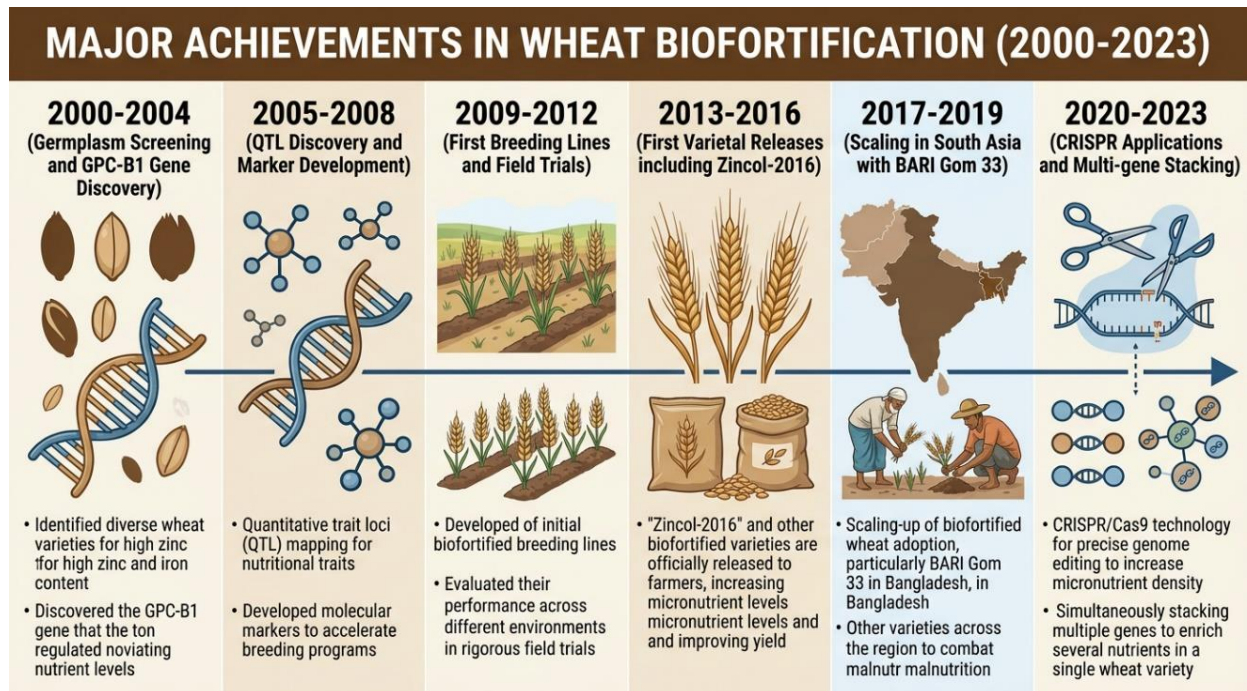
The nature of quality characteristics posed some specific difficulties to biofortified wheat as zinc and iron levels are too great to influence the dough properties and baking quality in terms of interactions with gluten proteins and oxidizing agents. Selective breeding and cross breeding however could keep the quality of processing at fairly commercial levels. Zincol-2016 and other varieties tested found that high zinc levels did not negatively impact the dough strength, extensibility or loaf volume compared to conventional varieties and was thus edible to both the baker and the consumer.

The breeding development cycle was conventional with the initial crosses and the introduction of the varietal at 8-12 years old comprised: screening of the germplasm, cross, screening, selection, multi-

environmental testing and regulatory approval. This extended breeding cycle ensured that breeding programs have to put in long-term investment and dedication but resulted in varieties that could be commercialized immediately after breeding without additional regulation and consumer acceptance issues that would arise with genetically modified crops. The practice achieved throughout the years of Harvest Plus programs show that even the traditional breeding can achieve target levels of nutrients and come up with varieties that can meet the requirements of the farmers, not to mention the nutritional benefits to the consumers.

However, natural limits exist to the use of

traditional breeding strategies. The natural genetic variability of the grain iron content compared to zinc was lower and the majority of breeding increased the content of iron only slightly. In addition, a majority of the zinc and iron present in the wheat grains are stored in the bran and aleurone layers that are usually removed by the miller to get white flour. The potential of traditional breeding to enhance the endosperm-specific distribution of the mineral was a major limitation in the use of conventional breeding methods since most of the world food production (more than 70 percent of the wheat consumed) is in refined flour form, and thus the use of complementary genetic engineering strategies was necessary.



Molecular and Genomic Approaches

Bio fortification of wheat has been transformed by molecular and genomic technologies which enable a tight control and manipulation of the genes which determine the amount of zinc and iron in the grain. The complexity of the wheat genome including the hexaploidy nature (AABBDD) and the sheer size of the genome (17 Gb) was initially not permissive of the study of the genome using molecular methods but, with the development of the sequencing technology and bioinformatics, it was progressively overcome, offering a potent tool to develop biofortified varieties (Kaur et al., 2024).

Genome-wide association studies (GWAS) have identified hundreds of loci to be associated with the grain zinc and iron concentration and can be used to select effectively and candidate genes to validate

the functions. A GWAS of wheat elite lines and landraces (n=380) conducted by Govindan et al (2022) identified 42 SNPs with significant effects on grain zinc and 38 SNPs with significant effects on iron, and a number of colocalized regions impacting both zinc and iron, suggest pleiotropy. Important groups of QTLs on chromosomes 2D, 6B and 7A explained 8-12% of the phenotype variation and were equivalent in their effects in various environments. These reports have shown that grain mineral concentration is a quantitatively inherited trait that is governed by a large number of small-effect loci, and not major genes, which makes it likely that genomic selection methods are needed to achieve optimal breeding results.

Chromosome	Position (cM)	Trait	Effect Size (mg/kg)	Candidate Gene	Function	Reference
2A	85.4	Zn, Fe	+4.2, +5.1	TaZIP4	Zinc transporter	Velu et al., 2018
2D	112.7	Zn	+5.8	TaNAS3	Nicotianamine synthesis	Govindan et al., 2022
4A	45.3	Fe	+6.2	TaVIT2	Vacuolar iron transport	Singh et al., 2022
5B	98.5	Zn, Fe	+3.9, +4.7	TaYSL16	Phloem transport	Hao et al., 2023
6B	67.2	Zn	+4.6	TaGPC-B1	Nutrient remobilization	Uauy et al., 2006
7A	123.8	Zn	+5.3	TaZIP7	Zinc uptake	Kumar et al., 2024
7B	56.4	Fe	+4.9	TaFRO1	Ferric reduction	

Note: Effect sizes represent additive effects in elite genetic backgrounds

Molecularly characterized candidate genes have been identified that have provided a clue into the mechanism of accumulating minerals in the grain. The TaZIP gene family (Zinc-regulated transporter, Iron-regulated transporter-like Protein) consists of a number of various sub-members and these are expressed in various tissues and stages of development, and are involved with zinc uptake and transportation. ZIP4 and ZIP7 are highly expressed in roots and developing grains, and have QTL effects on grain zinc concentration. Similarly, the movement of iron-nicotianamine complexes is mediated by Yellow Stripe-Like (YSL) transporters through the phloem and YSL16 is strongly correlated with content of grain zinc and iron. Genomic selection (GS) practices have become an effective breeding biofortification method where the breeding value based on genome-wide marker information can be predicted, without necessarily phenotyping of all selection targets. Rutkoski et al. (2016) developed that GS could increase genetic gain of grain zinc concentration by 20-30% in comparison to conventional phenotypic selection in wheat breeding programs. It is specifically applicable with moderately heritable characteristics that are expensive to phenotype, like mineral analysis that needs special equipment and would be a major

bottleneck in breeding pipelines. Application of GS to CIMMYT breeding programs of wheat has supplemented the genetic gain in zinc absorption without reducing the yield and other agronomic traits.

The transcriptomic and proteomic research work has arrived at regulatory networks that control grain mineral accumulation with the progression of development. The analysis of gene expression in grain filling showed that there is a co-ordinated rise in the expression of the genes of metal chelator synthesis (NAS, NAAT), transporter genes (YSL, ZIP) and senescence and nutrient remobilization regulating transcription factors. It was also established that the Gpc-B1 gene expressing the NAM-B1 transcription factor can be considered as a master regulator that triggers the expression of numerous nutrient remobilization-related genes and transcriptomic analysis revealed that the active alleles of the gene trigger early gene expression of senescence-related genes and transporter genes during grain development (Cantu et al., 2011).

Synteny-based comparative genomics methods that employed rice and other model species were used in the discovery of candidate genes. Such high levels of synteny between the wheat chromosome group 2 and rice chromosome 7 made it possible to

identify zinc transporter genes by using comparative mapping. This technique came in particularly handy in wheat whose genetic complexity had been a hindrance to efforts to clone genes. The 2018 release of the completely annotated wheat reference genome (IWGSC, 2018) went even further to enhance the molecular research and consequently enable the identification of candidate genes behind the QTL and the performance of functional validation experiments.

The problem that relates to antinutritional substances, which also affect the bioavailability of minerals, has been addressed using molecular strategies. Phytic acid (myo-inositol hexaphosphate), an interaction of minerals in the intestinal tract with phytic acid, is the primary blocker of zinc and iron absorption in wheat-based diets, which fails to allow absorption of minerals. Phytic acid can be found naturally in wheat grains, 8-12 g/kg, with a concentration that is located primarily in the aleurone layer. The molecular research revealed the inositol phosphate kinase gene (TaIPK1) as the last step in the production of phytic acid, which could be used as a target of molecular interventions to decrease the level of phytic acid (Ibrahim et al., 2022).

The use of molecular tools in breeding

programs has transformed radically the bio fortification of wheat with more specific and efficient selection and providing candidate genes to genetic engineering approaches. But molecular technologies also demonstrated that there is a genetic complexity in the accumulation of the grains, suggesting that gaining target levels can be attained by manipulation of a variety of genes but not by a monogenic solution. This discovery has led to the formulation of genomic selection methods and multi-gene stacking methods that are a combination of QTL and transgenes to give a cumulative effect.

GENETIC ENGINEERING STRATEGIES

Genetic engineering has proved to be a useful supplement to the conventional breeding in the sense that it provides access to new genetic variation and it is now more practical to achieve endosperm-specific biofortification, overcoming constraints of conventional methods. Transgenes have proven to accumulate zinc and iron in amounts vastly higher than conventional breeding and in particular of boosting the nutrient levels in a given fraction of the crop, the endosperm fraction utilized as white flour (Balk et al., 2022).

Genetic engineering is also being rationalized by the fact that there are a couple of flaws in natural breeding. Firstly, the genetic variation

in endosperm-specific mineral retention is a limited phenomenon because most of the micronutrients will be found in endosperm-tissues (aleurone and embryo) which have not been milled. Second, the traditional breeding has much cross breeding to restore the elite genetic backgrounds, increasing the development cycles. Third, the conventional breeding technique of stacking several advantaged alleles is more and more challenging with the number of loci. To overcome these limitations, genetic engineering is employed to enable the insertion of new genes or control factors causing the redirection of the nutrient storage in tissues of interest or increase the level of minerals.

One of the best methods of genetic engineering, which may be employed to biofortify wheat is nicotianamine synthase (NAS). NAS stimulates the production of nicotianamine, the major chelator that helps in the zinc and iron transportation in the phloem. Singh et al. (2022) generated lines of wheat containing the rice NAS2 gene that had constitutive promoters who achieved 40-60 percent enhancements in the level of zinc in the grain and 30-45 percent enhancements in iron. More importantly, the technique increased endosperm-specific concentrations

by 50-70 which is a crucial limitation of conventional breeding. In a few of the sites, field tests had a consistent expression of the trait and no punishment in yields and the nutrient levels had been maintained at a high level even in changing environments.

Another potential genetic engineering target has been the TaVIT2 (vacuolar iron transporter) gene. Although VIT2 plays the normal role of vacuolar iron sequestration which lowers the concentration of iron in the endosome, iron can be targeted to the endosome instead by modifying VIT2. In order to enhance iron fortification of the white flour, Beasley et al. (2022) used endosperm-specific promoter to express TaVIT2 which increased the amount of iron in white flour by 2.3 times in comparison with the common forms. This strategy in combination with constitutive NAS overexpression had even more impressive effects, and the iron and zinc concentration in white flour increased by 3.1 and 1.8-fold respectively, the highest endosperm-specific mineral concentrations recorded in wheat ever.

Construct	Promoter	Grain Zn (mg/kg)	Endosperm Zn (mg/kg)	Grain Fe (mg/kg)	Endosperm Fe (mg/kg)	Yield (% of control)	Reference
OsNAS2	Ubi-1	52.3	18.7	58.4	22.3	98	Singh et al., 2022
OsNAS2 + TaVIT2	Endosperm	48.7	16.2	92.6	68.4	96	Beasley et al., 2022
HvNAS1 + TaVIT2-D	Constitutive	54.1	19.8	87.3	58.7	97	Connorton et al., 2022
TaZIP4	Endosperm	46.9	15.4	51.2	18.6	99	Kumar et al., 2024
RNAi-TaIPK1	Ubi-1	42.3	14.8	63.7	24.8	95	Ibrahim et al., 2022
Control (non-GM)	—	32.5	9.2	42.8	15.3		

Endosperm values represent white flour fraction after standard milling

Ferritin over-expression is yet another alternative of augmenting the amount of iron in wheat. Plant ferritins are iron-binding proteins, in which the iron is stored in non-toxic and bioavailable forms. Drakakaki et al. (2005) in wheat were in a position to re-express soybean ferritin with the endosperm-specific promoters and found that there was 2-fold increment in the grains iron content. However, later research found out that a lot of the extra iron was in the bran fraction, which restricted its nutritional value to the consumers of refined flour. Other more recent approaches include ferritin expression with modified transporter expression to get the iron to the endosome with even greater

radical nutritional advantages.

Bioavailability is also solved by genetic engineering by reducing antinutritional compounds. Ibrahim et al. (2022) used RNA interference (RNAi) to suppress the expression of TaIPK1, the important enzyme in the phytic acid biosynthesis. The cell assays on lines resulted in 45-65% grain phytic acid content reduction and 25-35% zinc and iron bioavailability. Instead it is an augmentation process of nutrition and not an increment of the concentration of the mineral which will produce greater good per unit of mineral intake.

The technological advances that give rise to the prospect of genetic engineering in wheat

should be paid special attention. The regeneration potential of the genotype was genotype-specific and was associated with the hexaploid genome rendering wheat recalcitrant to transformation which was a hindrance to transgenic research in the past. Production of transgenic line of wheat has however become order of the day as transformation guidelines of some of the genotypes, and that is, the spring wheat line of Bobwhite, has been successfully produced. Both biolastic transformation with the use of particle bombardment and *Agrobacterium*-mediated transformation are effective, but the latter generates less intricate patterns of integration and more resilient inheritance. Genetic engineering of wheat has two major challenges namely, its regulatory approval and acceptance by the consumers. As opposed to the other major crops, where transgenic varieties have been extensively modified, transgenic wheat varieties have been commercial rejected to be used as human food in the major markets out of fear of consumer retaliation and market penetration. This is a regulatory fact that has limited transgenic bio fortified wheat application even though it has been established to be technically successful. Research has therefore been informed in the formulation of protocols that can be

transferred through the regulatory regime in the evolving guidelines like the genome-editing technology using CRISPR/Cas9 that is subject to numerous regulatory provisions in most countries.

The conventional and transgenic approaches are present to provide supplementary approaches of biofortification of wheat. Unlike the case with the traditional breeding where a variety can be obtained instantly and introduced in any market, genetic engineering can offer greater nutrient content and can be endosperm-specific modification that would enable to overcome the natural shortcomings of the traditional methods. The strategies are developed based on the regulatory conditions, tastes of the consumers and even based on certain nutritional objectives. Practically, the latter two methods will aid in the comprehensive bio fortification initiative whereby traditional varieties will have immediate effect and transgenic research will yield better technologies which can be integrated later on when regulation models are changed.

CRISPR/CAS9 GENOME EDITING

The latest and possibly radical differentiating technology innovation in biofortification of wheat is CRISPR/Cas9 genome editing since it allows a specific change of particular genes without the inclusion of foreign DNA

sequences that define the standard practice of genetic engineering. The difference has considerable regulatory classification and consumer acceptance impacts because different jurisdictions move towards regulatory systems that differ between genome editing and transgenic modification. Applications of CRISPR/Cas9 in wheat biofortification have evolved at a quicker pace between demonstrations of concept and field tests, showing that the technology might allow faster breeding advancements and attain new nutritional improvements (Li et al., 2021).

CRISPR/Cas9 system works based on a guide RNA (gRNA) which targets a specific target DNA sequence and the Cas9 endonuclease that induces a double-strand break at the targeted location. The cell repair system then closes the crack, usually with very minimal insertions/deletions which cause dysfunction of genes. In wheat biofortification, the technology consists of knocking out the genes that activate the

mineral accumulation or its bioavailability and the main target gene currently is TaIPK1 that produces phytic acid (Ibrahim et al., 2021).

TaIPK1 editing strategy is one approach to overcome a severe nutritional limitation of wheat, low mineral levels, by modulating phytic acid levels. Admittedly, whereas raising the concentration of the mineral, as with the methods, increases the concentration of phytic acid, reducing the concentration of phytic acid makes the remaining minerals more readily absorbed, which can be more nutritious in a number of grams consumed. CRISPR/Cas9 was used to induce TaIPK1 knockout lines in Ibrahim et al. (2022), and 50-70% reduction in grain phytic acid and 30-40% increase in zinc and iron bioavailability was determined using an in vitro digestion assays and by feeding human. It should be mentioned that these changes did not bring about a change in total mineral content but tremendously raised nutritional value.

Target Gene	Function	Editing Strategy	Phenotypic Effect	Bioavailability Increase	Field Performance	Status
TaIPK1	Phytic acid synthesis	Knockout	60% PA reduction	+38% Zn, +42% Fe	98% yield	Field trials
TaIPK2-2B	Phytic acid synthesis	Knockout	45% PA reduction	+28% Zn, +31% Fe	99% yield	Greenhouse

TaMIP S	Phytic acid precursor	Knockout	35% PA reduction	+22% Zn, +25% Fe	97% yield	Lab phase
TaVIT2 -D	Vacuolar iron transport	Knockout	Fe redistribution	+28% endosperm Fe	95% yield	Field trials
TaNAS 3	NA synthesis	Activation	+18% grain Zn	+15% Zn, +12% Fe	97% yield	Greenhouse
TaZIP4	Zinc transport	Activation	+22% grain Zn	+20% Zn	98% yield	Field trials

PA = phytic acid; NA = nicotianamine; Status as of 2024

The CRISPR/Cas9 multiplexing potential allows editing many genes simultaneously, which opens the prospects of obtaining synergies in nutrition. At the same time, the transfection of three genes (TaIPK1, TaVIT2, and TaNAS3) led to the reduction of phytic acid in a synergistic effect and an increase in the mineral concentration in grains in a synergistic effect. The resulting lines exhibited a reduction in phytic acid 55 percent, an increase in zinc 30 percent and an increase in iron 40 percent which could be more cumulative in multiple edits than in single-gene edits. This genetic stacking of editing as opposed to traditional breeding is a great benefit, as breeding can take 8-12 years to be completed but only 2-3 years with editing.

The fast-technological development of the instrument of transferring the components of CRISPR/Cas9 to wheat has taken place. Initially, transfection of both cas9 and gRNA

expression cassettes in a stable manner was done, and the transgenic lines of the alien DNA sequences were obtained. This is a good approach though it exposes the resultant lines to transgenic control measures. Even more recent developments are taking advantage of the transient expression of CRISPR/Cas9 ribonucleoprotein complexes into protoplasts or immature embryos and can lead to the generation of edited plants without introducing foreign DNA. This type of non-transgenic editing that does not involve the use of DNA might be commercialized in the new regulatory frameworks (Chen et al., 2023).

The issue that unites all the technologies of genome editing is the issue of off-target effects, which have been thoroughly studied in wheat CRISPR/Cas9 studies. Bioinformatically designed guide RNAs have been reported to have insignificant off-target mutations in whole-genome

sequencing of edited lines to specific sequences in the wheat genome. Polyploidy of the wheat has offered an additional protection since mutations in a single copy of the genome would have been counterbalanced by an active homoeolog, and such detrimental impacts would be unlikely to occur. However, it is important that stringent screening and validation of the edited lines is done to provide specificity and any unintentional changes are detected.

CRISPR/Cas9 edited crop regulatory frameworks are still developing around the world, and have important implications on wheat biofortification. The U.S department of agriculture (USDA) has ruled that the edited plants are not modified genetically engineered organisms and thus does not require regulation as it has not been incorporated with foreign DNA. Stricter regulations have been enforced by the European Union that has classified majority of genome editing applications as GMOs, which can be changed by policy review that is a continuous process. The establishment of regulatory systems in developing nations, where biofortified wheat may be most useful, is being built on a case-by-case basis, and certain nations are already ready to have a more optimistic attitude towards edited crops than transgenic ones (Kumar et al., 2022).

The fact that CRISPR/Cas9 technologies are rapidly developed to be applied in the biofortification of wheat means that the edited varieties can become available to farmers in 5-7 years after the first studies, which is significantly faster than the time-scale of traditional breeding. Several TaIPK1 lines have been successfully subjected to a greenhouse and confined field experimentation and demonstrated to be stable in their hereditary features and can be considered as acceptable agronomic with over one generation of progeny. This development leaves CRISPR/Cas9 at the head of a stop-gap measure towards the provision of healthier nutritional types of wheat, especially in those nations, which have favorable regulatory environments.

Combination of the traditional breeding, genetic engineering and genome editing will offer a complete set of tools with which to achieve the wheat biofortification. Although none of the conventional types can be deemed devoid of its advantages, the CRISPR/Cas9 technology is an innovation on the inherent shortcomings of the previous methods. The regulatory advantages of the technology such as speed and precision make it a game changer in the world in its effort to bio-enrich their food stuffs and can be used not only on wheat, but also on other staple

food crops which are facing a menace of deficiency of micronutrients.

AGRONOMIC BIOFORTIFICATION PRACTICES

Agricultural biofortification supplements genetic interventions, or the application of regimens of agricultural fertilizers in order to boost concentrations of micronutrients in edible crop fractions, which provide instant, flexible interventions, which can be applied to existing systems of production. In contrast to breeding strategies which demand the development cycles of years, agronomic biofortification can have an immediate deployment, providing solutions in the short term when genetically enhanced varieties are being developed and distributed (Cakmak, 2008).

The bio-enrichment of wheat with zinc has been actively sought after, basing on an established history of research on the impact of zinc deficiency in the world food crop sector. The foliage-applied zinc during the most crucial growth stages, especially between booting and early grain filling is by far more effective in zinc retention in the grain, than that of zinc in the soil. Zinc sprayed over the leaf at grain filling can be directly taken up into the growing grains by

the phloem with enrichment efficiencies of 30-50% compared to 5-10% when zinc is applied to soil. Wang et al. (2024) report a global meta-analysis of 156 studies that showed that at the rate of 2-4 kg Zn/ha, foliar zinc application could raise the grain zinc concentration on average by 28 percent without yield loss.

Biofortification of the iron in agriculture is less effective due to ease of oxidation and soil system immobilization of the iron and less effectiveness of the plants to take in the iron. Despite the fact that foliar iron application can potentially increase grain iron levels, the increase is typically on the order of 8-15 percent as compared to 20-30 percent when it comes to zinc. Chelated iron preparations, in particular ethylenediaminetetraacetic acid (EDTA) or citric acid, are more efficient in uptake, but are far costlier, limiting its use in developing country settings. Agronomic iron biofortification is not, then, the primary, but an additional step towards fighting iron deficiency.

Approach	Development Time	Cost per Variety	Zinc Increase	Iron Increase	Bioavailability	Adoption Barrier	Status
Conventional Breeding	8-12 years	\$2-3 million	+40%	+20%	Moderate	Low	Deployed
Marker-Assisted Selection	7-10 years	\$2.5-3.5 million	+45%	+25%	Moderate	Low	Deployed
Genomic Selection	6-9 years	\$3-4 million	+50%	+30%	Moderate	Low	Scaling
Transgenic (NAS)	5-8 years	\$4-6 million	+60%	+40%	High	High	Confined trials
CRISPR/Cas9	3-5 years	\$3-5 million	+55%	+45%	High	Moderate	Field trials
Foliar Zn Fertilizer	Immediate	\$15-25/ha/year	+28%	+5%	Moderate	Low	Deployed
Combined (CRISPR + Fertilizer)	3-5 years	\$3-5 million	+75%	+55%	High	Moderate	Development

***Zinc and iron increase relative to conventional varieties (baseline: 32 mg/kg Zn, 42 mg/kg Fe) ***

One such method is a mixture approach of genetic and agronomic method, which is zinc fertilizers coating on seed. The zinc-coated seeds will ensure that the zinc is easily absorbed by the plant during the initial stages and a well-formed root system will be developed that will be in a position to absorb the nutrients as the plant matures. Pakistan and Indian studies indicated that seed coating levels of zinc (2-4 g Zn/kg seed) increased zinc level in the grain by 12-18% and reduced the necessity to utilize the foliar application of zinc by 30-40%. This will lower the cost of application and will be evenly distributed, which is most likely to be embraced by smallholder farmers.

The time of zinc application has a great effect on the effectiveness of biofortification. The use of stem elongation and booting has been known to provide zinc to vegetative growth and anthesis and early grain filling applications which directly influence the accumulation of zinc in the grain. Split applications that provided 50 percent of the total amount of zinc in the bolting process and 50 percent in the early grain filling stage had a higher enrichment of zinc in the grains than single applications at each stage by 15-20 percent. With this time optimization, farmers are also able to optimize the maximum value of the nutrient without reducing the yield performance.

Combination of agronomic and genetic biofortification approaches is synergistic as opposed to additive. Highly-zinc content breeds of wheat are the result of traditional breeding, and absorb more zinc when treated with applied fertilizer, and absorb more zinc when treated with the same amount of applied fertilizer than conventional ones. This synergy implies that the most viable biofortification needs to be based on genetic and agronomic solutions in a way that the zinc-enriched types will accumulate more of the zinc to be added as a fertilizer and produce more final grain contents.

Soil management practices are influential on biofortification of zinc, which is highly affected by the management practices. The first predictor of the zinc status is the pH of the soil in which the extremes of the status of zinc deficiency can be found in the calcareous and alkaline soil since the zinc is cemented as insoluble compounds. Utilization of organic matter, especially farm yard manure or compost, chelation and diminution of fixation enhance the availability of zinc and the quantity of grain zinc grows by 8-12% with no extra zinc fertilization. On the same note, the application of phosphorus does not allow the zinc deficiency to occur even when excess phosphorus-zinc antagonism which is a

normal occurrence in the highly-managed wheat systems occurs.

Economic analysis reveals that the agronomic biofortification provides very cost effective interventions at cost of 0.30-0.50 per person per year to achieve considerable zinc intake benefits as compared to 3-5 per person per year in supplementation programs. This low cost and the fact that farmers already understand how to apply fertilizers and that such an approach could be immediately implemented makes agronomic biofortification a viable option in a scenario of short-term interventions that genetic versions of biofortification are being created and approved.

Yet, whether agronomic strategies which are to be introduced are sustainable is a question. Interventions that are based on fertilizers rely on the availability of continuous inputs and the economic ability of farmers to buy and use fertilizers. A challenge to the adoption to remote or marginalized agricultural communities can be market entry issues. Ecological issues such as the heavy metals accumulation in the soils and the possibility of ground water poisoning should be monitored, but, the safe dosage of zinc use has the least impact on the environment at the recommended doses. Lastly, agronomic biofortification is focused on zinc, instead of

iron, and therefore will require some complementary strategies to achieve a wholesome enhancement of micronutrients. The combination of genetic biofortification varieties and agronomic practices is the most viable avenue towards sustainable wheat biofortification. The improvement of baseline by high-zinc varieties can be enhanced by agronomic practices and the strategies of optimizing fertilizer strategies are necessary to ensure high-zinc varieties can achieve their full genetic potential. It is a hybrid approach, which can facilitate both short-term and long-term sustainability and provide a variety of entry-points to intervention in the multiple agro-situations and socioeconomic strata.

Bioavailability and Nutritional Impact

The fact that there is a higher amount of zinc and iron in the grains is not the sole aspect of a successful biofortification, but it is also necessary to make sure that the minerals are bioavailable in order to be absorbed in the human digestive system. The percentage of

the ingested nutrients that are breathed and utilized as physiological processes is the bioavailability. Nutritional parameters, which affect the bioavailability of the minerals are many, and zinc and iron absorption inhibitor in the wheat diets is the most potent phytic acid (Lowe et al., 2021). Having a normal content (8-12g/kg) of wheat grains, phytic acid complexes minerals such as zinc, iron, calcium and magnesium and the formed complexes are insoluble and thus cannot be absorbed or digested. Phytic acid to zinc molar ratio has been observed to be a good indicator of zinc bioavailability with ratios of less than 15 reported to be optimum ratios of absorption. The phytic acid: zinc proportions of the conventional varieties of wheat tend to be that of 25-35 which dramatically reduces the bioavailability to about 15-20. Better, as the rise in zinc is not accompanied by the fall in the phytic acid level, but the rise in zinc in biofortification, instead, prevents the additional zinc and does not allow it to be absorbed (Hall et al., 2024).

Strategy	Mechanism	Phytic Acid Reduction	Zinc Bioavailability Increase	Iron Bioavailability Increase	Sensory Impact	Status
Native phytase activity	Endogenous enzyme	0-5%	+8%	+10%	None	Natural variation
TaIPK1 knockout	Gene editing	50-70%	+35%	+40%	None	Field trials
Phytase supplementation	Added enzyme	60-80%	+45%	+55%	Minimal	Commercial

Fermentation	Microbial phytase	40-60%	+25%	+30%	Flavor change	Traditional
Soaking/germination	Phytase activation	20-30%	+12%	+15%	Texture change	Traditional
Amino acid chelation	Competitive binding	N/A	+18%	+22%	None	Research
Low-phytate varieties	Conventional breeding	15-25%	+10%	+12%		

Bioavailability increases relative to conventional wheat products

Research based on human intervention provides ultimate evidence of nutritional impact of bio fortified wheat. Sarawak et al. (2018) study in India was a cluster-randomized control trial, which provided 5-12-year-old children with six months of zinc-enriched wheat (38 mg/kg Zn). The means of Plasma zinc content increased significantly in the intervention group (+15%) and prevalence of zinc deficiency decreased significantly in the intervention group (vs. control groups fed on traditional wheat). Better still, children who were fed on biofortified wheat were found to have 17 fewer days of morbidity to typical illness like pneumonia, diarrhea and vomiting and were directly health benefiting on top of biomarker benefits.

A similar efficacy trial was carried out by Lowe et al. (2022) in rural Pakistan in adult women, the participants of which were supplied with zinc-biofortified wheat flour in 20 weeks. Biofortified wheat had significant

increases in plasma zinc concentration and exchangeable zinc pools in women who were consuming it in comparison to controls. The experiment demonstrated that bio enriched wheat would be capable of providing 30-40 percent of the daily amount of zinc intake via normal wheat consumption habits, which was biofortification and was found to be nutritionally sufficient to the extent desired by Harvest Plus.

The bioavailability of iron in biofortified wheat is more challenging to measure due to the chemical properties of iron and interaction with inhibitors. By assessing the high-iron wheat varieties on stable isotope methods, Haas et al. (2016) could compare the iron absorption levels and found that doubling the amount of iron in grain only led to doubling of iron absorption levels since phytic acid content also doubled in parallel. This fact explains why it is necessary to concentrate on the joint actions to fight mineral concentration and inhibitor levels in

order to reach the optimal nutrition effects. Bioavailability of biofortified wheat zinc and iron are largely affected by the processing methods. Phytic acid was broken down by phytase enzymes through the traditional fermentation of leavened bread by either using a yeast culture or sourdough culture to multiply bioavailability of minerals by 25-35%. The increased fermentation period, low PH and temperature, and sourdough fermentation is the most effective in increasing phytase activity. Without genetic modification it is possible to significantly enhance the nutritional value of these vintage ways of processing and offer culturally suitable avenues to the exploitation of the benefits of biofortified varieties.

The methodologies of bioavailability assessment are also improved and the simple in vitro assays of digestion are replaced with the complex in vivo experiments with the help of stable isotope tracers. The rapid identification of biofortified wheat lines on their mineral bioavailability can be done through Caco-2 cell culture models simulating the human intestinal absorption, before investing in the expensive human research. Such cellular models are very consistent with the human absorption data, and provide useful breeding program tools. Human volunteers have been used to study

stable isotopes and the results are conclusive as far as the efficiency of the absorption is concerned but are expensive and complex to use in large scale.

Both interaction and impact of nutritional action of the dietary diversity and intake of bio-fortified wheat also interrelate with each other. When wheat is a staple dietary constituent (the percentage of calories is 70-80) and when the dietary constituent is so substantial (the biofortification is very low) biofortification can be of great nutritional use in the population. But with the increase in the dietary diversity the ratio of biofortified wheat in the overall intake of the micronutrients reduces. This correlation would imply bio fortification would be the most effective in regions that exhibit wheat dependency and micronutrient deficiency is the worst and this is where Harvest Plus target geographies.

Cohort studies in the deployment areas are still in the stage of researching long-term health effects of the use of biofortified wheat. Early evidence shows that there is a long-term positive change in zinc status and reduction in morbidity among children that consume biofortified wheat on a regular basis. Health outcome based economical models depict a benefit-cost ratio of 15-25:1 of bio fortification programs, which is

significantly greater than a ratio of supplementation/food fortification program. These economic rewards, enhanced educational results and lowered medical expenses, cement the argument to forge on and increase biofortified wheat initiatives.

GLOBAL ADOPTION AND SCALING STRATEGIES

One of the most urgent issues that the biofortification programs face is the change in the direction of the research and development to mass production and implementation. Technical efficacy in high-zinc and high-iron wheat breeds offers sufficient though inadequate circumstances to obtain nutritional effect; effective delivery frameworks, farm uptake and customer acknowledgment are also important in minimizing micronutrient deficiencies on population levels (HarvestPlus, 2024).

Multi-scale was the strategy that HarvestPlus

employed to tackle the various limitation in the seed systems, value chains, policy frameworks and consumer awareness. The strategy appreciates that bio fortified varieties have to be competitive with conventional varieties in terms of agronomic performance, marketability, and quality of processing in addition to offering the extra nutritional advantage which motivates their adoption. Such a twofold need of not only competitive, but also nutritional performance requires a combined approach to technical, economic and social scaled dimensions.

Figure 2 shows the global location of bio-fortified varieties of wheat releases that are in progress and that the area covered by the varieties is growing at a more rapid pace with time starting with the original releases in South Asia and is currently encompassing 19 countries in Asia, Africa and Latin America.

Country	Year of First Release	Total Varieties Released	Area Coverage (000 ha)	Farmers Reached (000)	Adoption Rate (%)	Target Population (millions)
Pakistan	2015	7	785	425	12	45.2
India	2015	8	645	520	8	78.5
Bangladesh	2017	3	210	185	15	28.3
Afghanistan	2019	2	87	65	6	12.7
Nepal	2020	2	45	38	4	8.9
Ethiopia	2021	1	52	42	2	14.6
Mexico	2016	2	78	25	3	N/A
Bolivia	2018	2	34	18	5	

Adoption rate calculated as percentage of total wheat area in target regions

The scaling approach used by Harvest Plus has a few avenues based on various contexts

of the countries. In other countries where the system of a public sector seed system was

well established like in Pakistan and Bangladesh, the close liaison with national research and extension services to agriculture ensured that the dissemination of foundation seed to the public and the private seed producers was possible at an alarming rate. In poorer seed systems (such as Afghanistan and Ethiopia) community-based seed production systems that multiplied and distributed by communities of farm workers engaged in seed production were employed to produce local businesses, as well as to ensure access to seeds. These differentiated approaches recognized institutional differences between target countries whilst having the same technical standards of seed quality and purity of the varietal.

Its involvement of the private sector was a requirement in order to have size particularly in the countries where the commercial industries of seed were created. In Pakistan, collaborating with six major seed companies assisted in production and distribution of certified seed which could penetrate the market by making use of the existing dealer networks. The other similar alliances that included India, Bangladesh and Mexico leveraged the potential of the private sector to grow quality seeds, refine and sell them. However, with the introduction of the private sector, the introduction of intellectual

property rights was required and most of the biofortified varieties have been granted under a public domain or humanitarian licensing agreement to enable the smallholder farmers to utilize it.

The value chain development deals with the important bottlenecks among seed production and consumption. Other steps taken by HarvestPlus to create consumer awareness included multimedia campaigns and nutrition education programs and collaborating with health systems by building awareness of the nutritional benefits. The branding and quality certification system ensured that the bio fortified wheat products differed and enabled the farmers to obtain a price premium when selling the bio fortified wheat. Zincol brand got market recognition in Pakistan, and developed a market pull that encouraged the farmers to use this seed and was able to generate commercial seed production without subsidy.

Scaling enabling environments were created due to policy engagement on both national and international levels. Biofortification in national nutrition plans, food security plans and agricultural development plans incorporated biofortification as part of the bigger policy agenda and facilitated resources mobilization and institutional provisions. In Pakistan, biofortified wheat

was added to the national wheat procurement scheme that ensured that the product was able to be accessed in the market at high prices resulting in a considerable rate of adoption. Likewise, policy integration in Bangladesh, India and Afghanistan have allowed a continue scaling environment.

In economic terms scaling investments can be proved to be cost effective. By implementing in all the target countries with an initial investment of around 30-50 million, when embraced by over 1.2 million farmers, an estimated 6 million consumers had nutritionally-enriched wheat by 2023. Scaling investments are investment in development costs and delivery systems with 8:1 to 12:1 ratio of benefits to costs depending on the prevalence of the situation in the countries. These ratios are less than the breeding investments (because of the increased delivery cost), but very high compared with the benefit cost ratios of other micronutrient interventions.

There are still questions of scaling particularly with regard to long-term uptake by farmers when the subsidies have been phased out. It has been found through adoption research that the initial adopters, that is 15-20 percent of the farming populations, are larger and better endowed farmers with increased resources to

experiment with new varieties. To gain adoption among the remaining 80 it is required to address the constraints like lack of access to information, risks, and resource constraints. Farmer field school, extension services and demonstration trials were also effective to build the awareness and confidence though should be invested in the long-term to ensure continuity of adoption.

The scaling remains a problem with regards to quality control and maintenance of variety. In the genetic drift of seed multiplication, the concentration of the nutrients could reduce to lower concentration than it was intended particularly when the selection criteria are not strict when seed production occurs. Harvest Plus has put in place quality assurance measures whereby seed lots are periodically tested in terms of mineral concentration so that varieties that are delivered have nutritional targets. But the cost of implementation and the technical capacity necessitate implementation to be scaled down with community-based seed systems, especially.

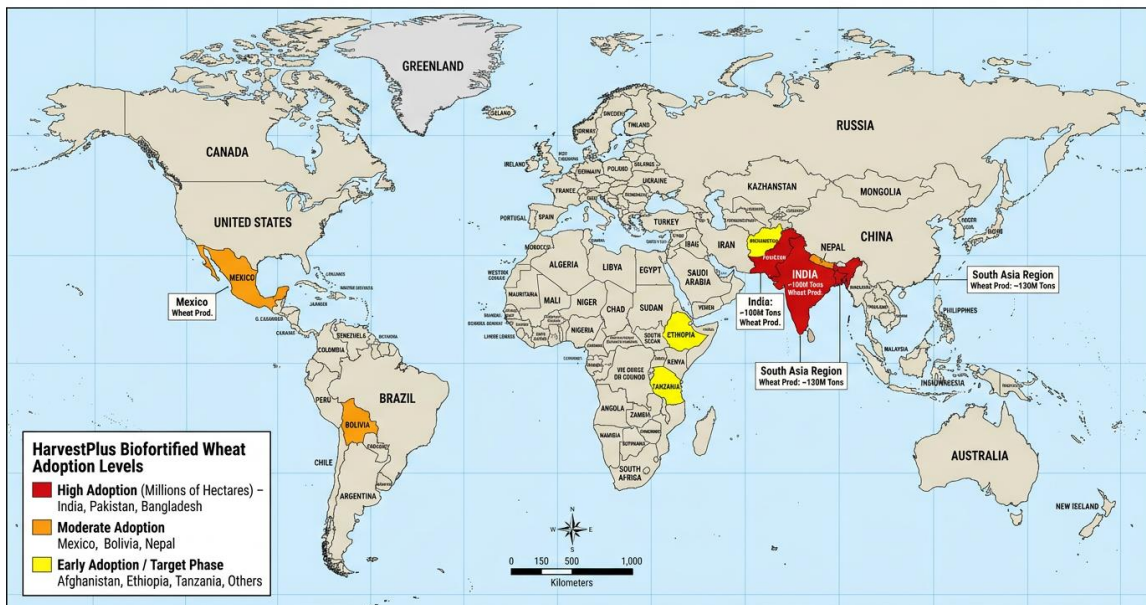
The desirable scaling is implied by the aspects of gender adoption. Women tend to be more influenced to buy bio fortified varieties in case they are made aware of the nutritional value of the product as they are the ones who prepare food at home and feed

children. In the majority of cultural contexts, however, women are not able to use bio fortified varieties because of the lack of control of agricultural decision making. Women communication intervention and other interventions that increase women involvement in agricultural decision making can increase the adoption level and the primary target beneficiaries benefit in the nutritional benefits.

Scaling strategies are more oriented to taking part in bigger scale agricultural development undertakings in the future than biofortification undertakings by itself. The

introduction of bio fortified varieties as part of climate-smart agriculture programs, sustainable intensification programs as well as value chain development projects is an added resource base as well as a way of ensuring that the consideration of nutrition is mainstreamed in the agricultural development. This form of integration is more sustainable and has a higher chance of reaching more individuals compared to project based biofortification processes although more inter-sector and inter-institutional coordination is required.

GLOBAL DISTRIBUTION OF HARVESTPLUS BIOFORTIFIED WHEAT VARIETIES (ILLUSTRATIVE DATA)



Climate Change Challenges

Climate change presents great challenges to wheat bio fortification which can erode the gains made by breeding and agronomic interventions. Increased carbon dioxide

concentration in the atmosphere, temperature and change of precipitation patterns have various physiological impacts on wheat growth, quality of wheat grains and mineral

content in grains. The knowledge of these effects is essential in coming up with climate-resistant biofortification technologies that can maintain nutritional gains in the future climate conditions (Sapkota et al., 2024).

These are the effects of a rise in the level of CO₂ in the atmosphere which is likely to increase to 550 ppm with the current level of emission which is fed directly into the mineral content of the wheat grain by the dilution of the carbohydrates. Myers et al. (2014) showed their findings of free-air CO₂ enrichment (FACE) experiments and discovered that the wheat plants that had been

grown in high CO₂ concentration (546-586 ppm) had a 9.3% lower zinc and 5.1% lower iron in them than those that had been grown under ambient condition. This dilution effect may be explained by the stimulated accumulation of carbohydrates in the grains that adds weight to the grains without any corresponding mineral contents. The possibility to locate genotype-specific variability in the extent of the reduction indicates that it is possible to breed biofortified varieties which are resistant to climate changes.

Climate Scenario	CO ₂ (ppm)	Temperature Increase (°C)	Grain Zn Change (%)	Grain Fe Change (%)	Protein Change (%)	Yield Change (%)
RCP 2.6 (Low)	440	+1.0	-5.2	-3.1	-4.5	+2.3
RCP 4.5 (Medium)	540	+2.0	-9.8	-6.2	-8.7	-5.1
RCP 8.5 (High)	670	+4.0	-15.6	-10.4	-13.2	-12.8
With Adaptation*	540	+2.0	+2.3	+1.8	+1.5	-3.2

Adaptation scenario includes improved varieties with elevated mineral concentration and biofortification

Climatic change is associated with rise in temperature which affects the concentration of micronutrients in wheat as far as growth is concerned and reduction in the grain filling period. Increased temperatures result in a reduction in time interval in which mineral is deposited in growing grains, resulting in a decreasing ultimate concentration with

increasing retention time, where the overall uptake remains fixed. Remobilization of vegetative tissues to grains is especially interrupted by heat stress during periods of critical grain filling as senescence processes are hastened and transport systems are ineffective. The combination of these developmental effects of high CO₂ and

effects of high CO₂ on zinc and iron concentration leads to model studies that assumed that all increases in mean temperature during grain filling would reduce the zinc and iron concentration by 3-5 percent because of these developmental effects.

Change in the frequency of the rainfall and occurrence of the most adverse weather conditions affects the supply of the micronutrients in the soil networks and is converted to the uptake of the micronutrients by plants and grains accretion. The drought stress limits the exploration of the origins and uptake of the micronutrients and excess precipitation may result in losses of the mobile nutrients in the root zones. They are highly dependent on the character of soil and the environment around that develops complex configurations of climatic influences on the mineral content of the grains produced within the production settings. Regions experiencing more droughts like most of the wheat-producing areas of South Asia and Sub-Saharan Africa are of particular concern as to whether the grain mineral quality will reduce in climate change.

Among the primary adaptation measures, breeding biofortified varieties with climate-resistance is one of the approaches. This plan will identify and breed varieties that will

retain high concentration of minerals with high stresses of CO₂ and temperature. The screening of the current biofortified varieties under climate control and FACE experiments can be used to identify less climate sensitive genotypes. At a very early stage there have been signs that some bio-fortified varieties, namely those which reach high levels of accumulation by increasing uptake, rather than by increasing accumulation, are more resistant to climate stress, in nutritional quality. With climate resilience being a definite selection criterion in breeding programs, it is definite that the future biofortified varieties will be beneficial in terms of nutrition in different environmental conditions.

The effects of the climate change on the mineral content of grain can be supplemented by implementing Agronomic adaptation to a certain extent. To guarantee absorption and retention of the micronutrients it might be appropriate to change the date of sowing to avoid heat stress during the grain filling and conservation agriculture to increase moisture retention in the soil besides timing and optimum use of the fertilizers. The significant rise in CO₂ concentration is particularly at the point of zinc fertilization being necessary since the effect of dilution can be somewhat offset by a higher overall uptake of zinc.

When used as a foliar spray on grain filling, zinc can be utilized effectively since it can be sprayed to counter any limitation mechanism that could be present in the soils and it would directly cause the accumulation of zinc in the grains.

The nutritional quality is determined by computer modeling of the various scenarios of climate change, adaptation strategies and socioeconomic factors to determine the best investment strategies to sustain the nutritional quality. The findings demonstrate that the mineral content of grains can be conserved or even enhanced during the climate change period through the incorporation of climate-resistant varieties with agronomic modifications in the middle of the moderate enhancement in the use of fertilizers. Nevertheless, only in the case of long-term investment in the breeding process, the availability of fertilisers, and extension services, such results are possible, which is why it is necessary to touch upon the climate adaptation as a smaller part of larger agricultural development programs.

It is also possible that geographic change could occur in production of wheat and this poses more challenge to bio fortification programs. The biofortification delivery methods currently available may also have to be geographically modified as well as the

favourable sites of production are either relocating far northwards or up the mountain with the warming. Such dynamism requires the need to use flexible, mobile delivery strategies to the fixed location-based programs, which can increase the costs of delivery but ensure additional access to vulnerable groups as production trends vary. Climate change can also have an impact on the epidemiology of human micronutrient deficiencies which may change biofortification intervention priorities. Shifting patterns of diseases especially more occurrence of diarrheal diseases during extreme precipitation can worsen the loss of zinc and iron leading to the need to increase demands. Moreover, other food systems affected by climate can also decrease dietary diversity in at-risk populations where wheat contributes a bigger fraction of nutrient provision and biofortification contributes a bigger fraction of nutritional security.

The research priorities of climate-resilient biofortification are: mechanisms of genotype variation of climate sensitivity, high-throughput phenotyping to analyze mineral concentration in climate stress and agronomic practice. System approaches can also be applied through crop modeling, climate projections and socioeconomic analysis to determine priority areas and the

best intervention plans to enhance the maximum benefits of limited resources. International co-operation during such research work will ensure that the process will be accelerated and that bio fortification programmes will be successful even in the circumstances of climate changes that will be unveiled in the future.

FUTURE PERSPECTIVES AND CONCLUSIONS

In the last 2 decades, tremendous gains have been made in the field of biofortification of wheat with the conceptual approach being substituted by intervention that has been implemented on over 360 million people globally. The success stories of traditional breeding, the evolution of the molecular genetics and the future technology of genome editing meet at a crossroad with wheat biofortification and an opportunity to increase and exponentially with a greater nutritional product. Multiple approaches can enhance biofortification science in the future, enhancing the delivery modes, and increasing the coverage of the vulnerable groups (Kumar et al., 2022).

The breeding activity will be increased greatly with the emergence of genetic technology. Further breeding value prediction of mineral concentration and yield, which are already in CIMMYT wheat breeding programs, will be obtained through

genomic selection, which will lead to better selection of both breeding values. Increases in zinc concentration and iron concentration will be quicker with larger increases in reference populations and better prediction models, potentially with gains of 2-3%/year relative to the current gains. Genomic selection along with fast generation improvement (speed breeding) has the potential to save breeding times that might otherwise have been 8-10 years to 4-5 years and allow responding to new challenges and opportunities to be quicker.

The most radical type of technology that can be used in next-generation biofortification is CRISPR/Cas9 genome editing. Its ability to fine-tune genes controlling the synthesis of phytic acid, the capacity to vacuolarly trap and sequester nutrients and the capacity to move the nutrients without the necessity to insert the alien DNA is the solution to the major limitations of the previous approaches. Multifunction editing techniques focusing on a group of genes at once offers prospects of superior synergetic gains than that of a single gene. As a novel regulatory framework with the potential to provide a difference between genome editing and transgenic modification, it might be that the quality of the edited types will pass through the approval and acceptance of consumers more quickly,

accelerating the application to the countries of interest.

The future should be in the further development of biofortification to other limiting micronutrients. It has been estimated that there has been Selenium deficiency among 1 billion people in the world with a specific concentration in the areas where the soils are deficient in selenium. The deficiency of the provitamin A is still prevalent in the population that depends on the cereal based diet. Selenium is produced to a certain degree especially when the wheat is bred as it is a ready-to-absorb selenium in the soil and in the leaves. Metabolically engineering wheat to be biofortified with vitamin A is a more complex project, but a natural extension of the successful projects in other crops (orange-fleshed sweet potato and Golden Rice).

Climate change should be included in the breeding goals of biofortification. This maintenance of the stable mineral content at the heightened CO₂ and temperature stress conditions in the selection process guarantees delivery of nutritional value to future varieties with a change in the environmental conditions. Genetic processes related to climate sensitivity are well known and permit a certain degree of choice as to the climate resilience, and do not necessarily contradict

the interaction of mineral-accumulation and stress-tolerance regimes. Predictive models of environmental conditions and grain mineral quality have been developed to enable breeding to be done in particular target production environments.

The combination of digital agriculture technologies and systems of biofortification delivery opens the perspective of higher efficiency and effectiveness of scaling. Remote sensing can be employed to keep track of patterns of adoption and a greater area can be addressed than with self-reporting or sample survey. The mobile mobile applications can provide the farmers with tangible information on the variety's performance, management and nutrition benefits. The identity preservation and quality control value chains can be confirmed through blockchain technologies and allow premium prices of bio fortified products. These e-innovations save delivery expenses and enhance monitoring, assessment and accountability.

Nutritional enhancement plans should advance beyond merely concentrating on the levels of minerals to cover bioavailability and quality of the diet in entirety. More nutritional value per unit of consumed mineral is being generated through innovations in gene editing or processing of

reducing phytic acid. The fact that the populations are largely facing a combination of multiple deficiencies of micronutrients is overcome by conjoint fortification of a number of nutrients. Full nutrition security solutions consist of integrations of biofortification programs and programs on dietary diversification, food fortification where warranted and selective supplementation of severely deficient individuals.

Biofortification schemes require additional investment on breeding and delivery systems and setting up of mechanisms that would enable the schemes to be viable in the long run. Examples of public goods that may justify public investment are initial investments in bio fortified varieties. The delivery systems however ought to be handed over to greener financing programmes whereby the industrial sector must be involved, the government commitment and maybe a few cents of bio-enriched goods which can be proved to be safe. New funding mechanisms to commercialize biofortification can be identified by developing impact investment models which can be used to tie financial gain with nutrition outcome.

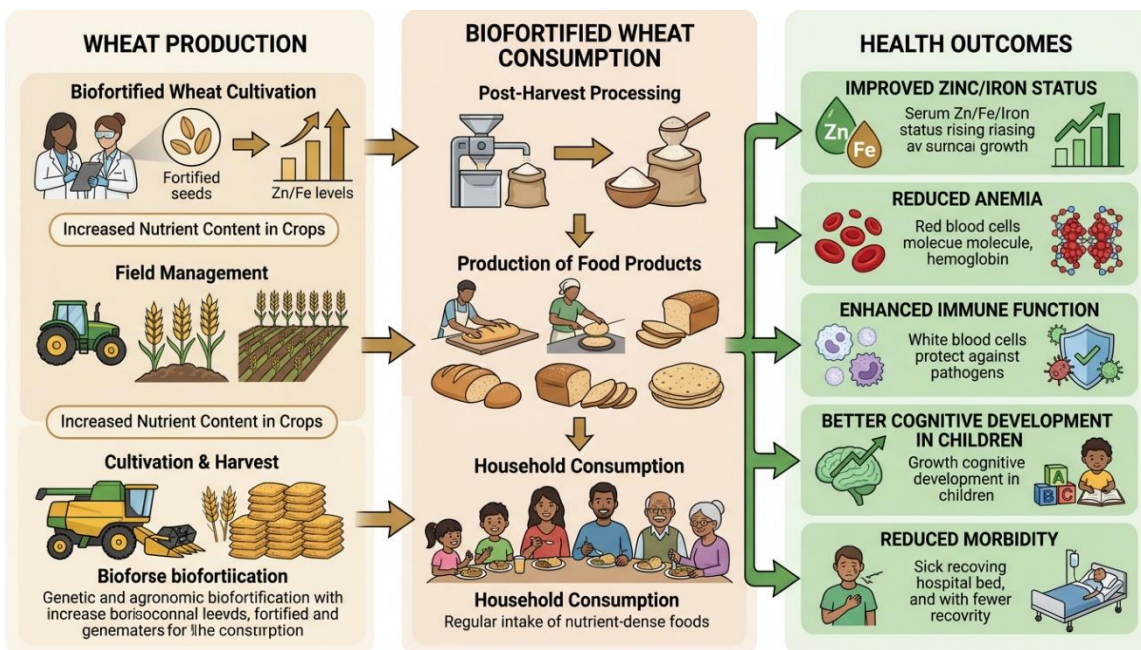
The international cooperation and exchange of knowledge is a quicker way forward in all

areas of biofortification. Biofortification Priority Index has been used to identify the highest potential countries and populations that can be targeted in the biofortification interventions hence providing the opportunity to strategically allocate resources. The CGIAR biofortification network helps in sharing of germplasm, inter-regional capacity building and learning. The contribution of South-South cooperation can assist the already developed bio-fortification programs of the developing countries to know the pioneers and to avoid their mistakes and accelerate the process. These types of networks of collaboration are not only improving the impacts of individual country investments but also best practices are easily disseminated across target areas.

It is possible to envisage a vision of holistic biofortification of wheat in the future that encompasses all types of wheat that are used in the deficient regions of the micronutrients can be fortified to the desired levels of the minerals, the bioavailability can be optimized by means of processing technologies and made accessible to the entire population. International organizations, governments and research institutions and business partners will have to invest long-term but the payoffs may be radical changes in the nutrition security of the world. The potential of

technology, economical demonstrations, and political determination offer even more possibilities to scale-up biofortification to the

Sustainable Development Goal targets in nutrition and health.



REFERENCES

Ali, M., & Borrill, P. (2020). Applying genomic resources to accelerate wheat biofortification [Review of Applying genomic resources to accelerate wheat biofortification]. *Heredity*, 125(6), 386. Springer

Nature. <https://doi.org/10.1038/s41437-020-0326-8>

Bhattacharya, S., Sathiyabalan, A., & Amir, M. (2024). Advancing Nutritional Quality through Genomic Approaches for Biofortification in Cereal Crops: A Review. *PLANT CELL BIOTECHNOLOGY AND MOLECULAR BIOLOGY*, 25,

110. <https://doi.org/10.56557/pcbmb/2024/v25i5-68713>

Borrill, P., Connorton, J. M., Balk, J., Miller, T., Sanders, D., & Uauy, C. (2014). Biofortification of wheat grain with iron and zinc: integrating novel genomic resources and knowledge from model crops. *Frontiers in Plant Science*, 5. <https://doi.org/10.3389/fpls.2014.00053>

C, R., Kumar, S., Ranjan, R., Kumhar, S. R., & Govindan, V. (2022). Genomic approaches for improving grain zinc and iron content in wheat [Review of Genomic approaches for improving grain zinc and iron content in wheat]. *Frontiers in Genetics*, 13. *Frontiers*

Media. <https://doi.org/10.3389/fgene.2022.1045955>

Chakraborty, B., & Shrivastav, S. P. (2024). Genomic Advances in Biofortification of Iron and Zinc in Wheat (*Triticum aestivum* L.). *Journal of Advances in Biology & Biotechnology*, 27(6), 9. <https://doi.org/10.9734/jabb/2024/v27i6861>

Devate, N. B., Krishna, H., Mishra, C. N., Manjunath, K. K., Sunilkumar, V. P., Chauhan, D., Singh, S., Sinha, N., Jain, N., Singh, G. P., & Singh, P. K. (2023). Genetic dissection of marker trait associations for grain micro-nutrients and thousand grain weight under heat and drought stress conditions in wheat. *Frontiers in Plant Science*, 13. <https://doi.org/10.3389/fpls.2022.1082513>

Gupta, O. P., Singh, A. K., Singh, A., Singh, G. P., Bansal, K. C., & Datta, S. K. (2022). Wheat Biofortification: Utilizing Natural Genetic Diversity, Genome-Wide Association Mapping, Genomic Selection, and Genome Editing Technologies [Review of Wheat Biofortification: Utilizing Natural Genetic Diversity, Genome-Wide Association Mapping, Genomic Selection, and Genome Editing Technologies].

Frontiers in Nutrition, 9. *Frontiers Media*. <https://doi.org/10.3389/fnut.2022.826131>

Gupta, O. P., Singh, A., Pandey, V., Sendhil, R., Khan, Mohd. K., Pandey, A., Kumar, S., Hamurcu, M., Ram, S., & Singh, G. P. (2024). Critical assessment of wheat biofortification for iron and zinc: a comprehensive review of conceptualization, trends, approaches, bioavailability, health impact, and policy framework [Review of Critical assessment of wheat biofortification for iron and zinc: a comprehensive review of conceptualization, trends, approaches, bioavailability, health impact, and policy framework]. *Frontiers in Nutrition*, 10. *Frontiers Media*. <https://doi.org/10.3389/fnut.2023.1310020>

Ibba, M. I., Gupta, O. P., Govindan, V., Johnson, A., Brinch-Pedersen, H., Nikolić, M., & Taleon, V. (2022). Editorial: Wheat biofortification to alleviate global malnutrition. *Frontiers in Nutrition*, 9. <https://doi.org/10.3389/fnut.2022.1001443>

Krishnappa, G., Khan, H., Krishna, H., Kumar, S., Mishra, C. N., Parkash, O., Devate, N. B., Nepolean, T., Rathan, N. D., Mamrutha, H. M., Srivastava, P., Biradar, S.

S., Uday, G., Kumar, M., Singh, G. P., & Singh, G. P. (2022). Genetic dissection of grain iron and zinc, and thousand kernel weight in wheat (*Triticum aestivum* L.) using genome-wide association study. *Scientific Reports*,

12(1). <https://doi.org/10.1038/s41598-022-15992-z>

Krishnappa, G., Rathan, N. D., Sehgal, D., Ahlawat, A. K., Singh, S. K., Singh, S. K., Shukla, R. B., Jaiswal, J. P., Solanki, I. S., Singh, G. P., & Singh, A. M. (2021). Identification of Novel Genomic Regions for Biofortification Traits Using an SNP Marker-Enriched Linkage Map in Wheat (*Triticum aestivum* L.). *Frontiers in Nutrition*, 8. <https://doi.org/10.3389/fnut.2021.669444>

Kumar, A., & Kaushik, P. (2023). Biofortification of Wheat Landraces: Recent QTL Mapping Advances. In *IntechOpen eBooks*. IntechOpen. <https://doi.org/10.5772/intechopen.111972>

Kumar, R., Malo, K., Lagoriya, D. S., Joshi, S., Patyal, D., Ali, I. H., Renuka, R., Singh, A., & Tutlani, A. (2025). Advancing Nutritional Quality through Genome Editing for Biofortification: A Comprehensive Review. *Journal of Advances in Biology & Biotechnology*, 28(10),

744. <https://doi.org/10.9734/jabb/2025/v28i103100>

Manjunath, K. K., Krishna, H., Devate, N. B., Sunilkumar, V. P., Chauhan, D., Singh, S., Mishra, C. N., Singh, J., Sinha, N., Jain, N., Singh, G. P., & Singh, P. K. (2023). Mapping of the QTLs governing grain micronutrients and thousand kernel weight in wheat (*Triticum aestivum* L.) using high density SNP markers. *Frontiers in Nutrition*, 10. <https://doi.org/10.3389/fnut.2023.1105207>

Nasim, A., Hao, J., Tawab, F., Jin, C., Zhu, J., Luo, S., & Nie, X. (2025). Micronutrient Biofortification in Wheat: QTLs, Candidate Genes and Molecular Mechanism. *International Journal of Molecular Sciences*, 26(5), 2178. <https://doi.org/10.3390/ijms26052178>

Saini, D. K., Devi, P., & Kaushik, P. (2020). Advances in Genomic Interventions for Wheat Biofortification: A Review. *Agronomy*, 10(1), 62. <https://doi.org/10.3390/agronomy10010062>

Sen, H., Kumar, A., & Janeja, H. S. (2024). Biofortification of Major Crops through Conventional and Modern Biotechnological Approaches to Fight Hidden Hunger: An Overview. *Journal of Advances in Biology &*

Biotechnology, 27(7),
96. <https://doi.org/10.9734/jabb/2024/v27i7970>

Sharma, D., Ghimire, P., Bhattarai, S., & Adhikari, U. (2020). BIOFORTIFICATION OF WHEAT: GENETIC AND AGRONOMIC APPROACHES AND STRATEGIES TO COMBAT IRON AND ZINC DEFICIENCY. *Sustainability in Food and Agriculture*, 1(1), 48. <https://doi.org/10.26480/sfna.01.2020.48.54>

Sharma, D., Ghimire, P., Bhattarai, S., Adhikari, U., Khanal, S., & Poudel, P. B. (2020). Biofortification of wheat: Genetic and agronomic approaches and strategies to combat Iron and Zinc deficiency. *International Journal of Environment Agriculture and Biotechnology*, 5(4), 1077. <https://doi.org/10.22161/ijeab.54.29>

Sharma, V., Choudhary, M., Kumar, P., Choudhary, J. R., Khokhar, J. S., Kaushik, P., & Goli, S. (2021). Harnessing the Wild Relatives and Landraces for Fe and Zn Biofortification in Wheat through Genetic Interventions—A Review. *Sustainability*, 13(23), 12975. <https://doi.org/10.3390/su132312975>

Sheera, A., Aftab, N., Tanwar, J., Meena, V. K., Kumar, O. P., Dey, T., Singh, V., Singh,

J., & Meena, R. K. (2025). Biofortification strategies for wheat: Enhancing zinc and iron nutritional quality to combat micronutrient deficiencies and ensure food security. *Journal of Food Composition and Analysis*, 147, 108009. <https://doi.org/10.1016/j.jfca.2025.108009>

Sun, M., Luo, Q., Zheng, Q., Tong, J., Wang, Y., Song, J., Zhang, Y., Pu, Z., Zheng, J., Liu, L., AnDing, Z., Rasheed, A., Li, M., Cao, S., Xia, X., He, Z., & Hao, Y. (2023a). Molecular characterization of stable QTL and putative candidate genes for grain zinc and iron concentrations in two related wheat populations. *Research Square (Research Square)*. <https://doi.org/10.21203/rs.3.rs-2858009/v1>

Sun, M., Luo, Q., Zheng, Q., Tong, J., Wang, Y., Song, J., Zhang, Y., Pu, Z., Zheng, J., Liu, L., AnDing, Z., Rasheed, A., Li, M., Cao, S., Xia, X., He, Z., & Hao, Y. (2023b). Molecular characterization of stable QTL and putative candidate genes for grain zinc and iron concentrations in two related wheat populations. *Theoretical and Applied Genetics*, 136(10). <https://doi.org/10.1007/s00122-023-04467-y>

Tadesse, W., Gataa, Z. E., Rachdad, F. E., El-Baouchi, A., Kehel, Z., & Alemu, A. (2023).

Single- and multi-trait genomic prediction and genome-wide association analysis of grain yield and micronutrient-related traits in ICARDA wheat under drought environment. *Molecular Genetics and Genomics*, 298(6), 1515. <https://doi.org/10.1007/s00438-023-02074-6>

Wani, S. H., Gaikwad, K. B., Razzaq, A., Samantara, K., Kumar, M., & Govindan, V. (2022). Improving Zinc and Iron Biofortification in Wheat through Genomics Approaches [Review of Improving Zinc and Iron Biofortification in Wheat through Genomics Approaches]. *Molecular Biology Reports*, 49(8), 8007. Springer Nature. <https://doi.org/10.1007/s11033-022-07326-z>

Wanyera, R., & Owuoché, J. (2017). Wheat Improvement, Management and Utilization. In *InTech eBooks*. <https://doi.org/10.5772/63694>