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Advancing Zinc and Iron Biofortification in Wheat: Current Breeding Approaches and Future Prospects for Pakistan

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Abstract

The micronutrient malnutrition is especially prevalent in Pakistan, of zinc (Zn) deficiency and iron (Fe) deficiency, as 97% of its population is dependent on wheat (*Triticum aestivum* L.) as calorie source. About 70 percent of soils in agriculture sector of Pakistan are low in plant available Zn. Soil micronutrient phytoavailability is significantly limited by highly calcareous and alkaline surface horizon of the Indus basin alluvium, enabling to directly pass on the soil micronutrient deficiencies to the food to be consumed by humans. By using landraces, wild relatives, and CIMMYT developed and supplied synthetic hexaploids and national partnerships, five biofortified cultivars have already been commercialized (Zincol-2016, Akbar-2019, Nawab-2021, Tarnab Rehbar, and Tarnab Gandum-I). Multiple markers assisted and genomic selection based introgression of micronutrient QTLs is possible without yield penalty, thanks to modern molecular frameworks to speed these fundamental improvements. At the same time, some new frontier biotechnologies like the overexpression of the *NICOTIANAMINE SYNTHASE* (NAS) gene or the suppression of phytic acid generation through a CRISPR/Cas9 approach provide solutions of major throw when the addressing plant nutrient bioavailability is concerned. The objective of high grain production and micronutrient density, as well as complicated genotype by environment (G × E) interactions driven by stress factors such as heat and drought, these are considerable challenges for the modern breeding. The use of saved seed from previous years is common. Overcoming these barriers will require speed breeding, high throughput phenotyping, and intensive testing in multiple environments to be integrated in a short time. Combining conventional, molecular, and biotechnological approaches to cultivate dual purpose crops capable of producing high biotic and abiotic stress tolerance contributes the most to managing systemic nutritional insecurity issues in Pakistan.
Keywords: CIMMYT, marker assisted selection (MAS), genomic selection (GS), CRISPR, calcareous soils, HarvestPlus

IntroductionWheat (*Triticum aestivum* L.) plays a pivotal role in Pakistan's food security, contributing about 72% of daily required calories, and providing the most important source of macro and micronutrients (Ishfaq et al., 2021). Almost 90% of varieties cultivated in Pakistan are of genetic heritage developed by the International Maize and Wheat Improvement Center (CIMMYT). The crop covers a harvested area of around 9 million hectares (Government of Pakistan & Ministry of National Food Security & Research, 2025). Despite wheat being the centerpiece of the national diet, the prevalent case of micronutrient malnutrition (MNM) is also alarmingly high. It is referred as "hidden hunger". Zinc Deficiency (ZD) is gaining momentum in Pakistan, with more than 60 million people affected and 18% of under fives and 37% of pregnant women considered at risk, with estimated loss of GDP of nearly USD 3 billion due to the high burden of malnutrition in Pakistan (Lowe et al., 2022).

Wheat biofortification with zinc and iron constitutes one of the most cost effective and sustainable approaches for addressing population level micronutrient deficiencies (Latha & Prakash, 2024). In contrast to agronomic fortification via fertilizer application which entails recurring input costs and is vulnerable to fixation in alkaline calcareous soils genetic biofortification offers a heritable, one-time solution that imposes no additional production costs on resource poor farmers (Azeem et al., 2025). Collaborative efforts among CIMMYT, HarvestPlus, and national agricultural research institutions have already enabled the release and widespread adoption of zinc enriched wheat varieties (Magallanes-López et al., 2017).

There are unique environmental challenges in Pakistan that can hinder efforts to develop biofortified wheat. Soils of Indus basin are mainly calcareous (pH 7.8-8.5) and alkaline with very low organic content (which is less than 1%), due to which the soils have limited Zn and Fe phytoavailability (Khan et al., 2023). Deficiency of Zn is the first micronutrient soil-level deficiency problem in Pakistani Agriculture that is affecting approximately 70% of the arable land with maximum 75% in southern Punjab (Maqsood et al., 2015). Improving the situation with the traditional Zn fortification of the soil is still highly inefficient because there is rapid adsorption to clay particles or precipitation in the form of $Zn(OH)_2$ and $ZnCO_3$ in such alkaline soils to a point that plants cannot absorb (Khan et al., 2023). The edaphic constraints require genetic engineering of the wheat cultivars to improve. Climate change caused by humans is worsening the nutritional quality of wheat in the South Asian region. A rise in temperature coupled with an increase in CO_2 in the atmosphere lowers grain zinc (Zn) and iron (Fe) concentrations that are a result of carbohydrate dilution and disruption of nutrient transporter mechanisms (Dwivedi et al., 2023). For every 1°C increase in temperature, there is a 10.5% decrease in grain Zn content (Bello et al., 2026). Pakistani wheat is grown in a highly heterogeneous agroecological region, ranging from temperate Potohar plateau to hot plains of Sindh and hence the selection of environmentally stable micronutrient phenotypes is a fundamental breeding priority.

Breeding Methodologies for Zn and Fe Biofortification

Conventional Breeding Approaches

Conventional breeding is the most common and cost effective approach towards biofortification, of which the primary, secondary and tertiary gene pools are characterized by diversity and utilized to increase the concentration of zinc (Zn) and iron (Fe) in wheat grain (Gupta et al., 2024). It relies on the effectiveness of this approach, which is based on the availability of strong genetic seed stocks with high levels of essential micronutrients and the ability to select for these traits without compromising other desirable agronomic traits.

Genetic Diversity in Wheat and Wild Relatives

Relative to its wild progenitors, cultivated bread wheat (*Triticum aestivum*) harbors restricted genetic diversity for grain micronutrient accumulation. Diploid progenitors of hexaploid wheat, including *Aegilops tauschii* (D genome donor), einkorn (*Triticum monococcum*), wild emmer (*T. dicoccoides*), *T. polonicum*, *T. spelta*, and landraces of *T. aestivum*, represent invaluable reservoirs of high Zn and Fe alleles. In contrast, wild wheat accessions, notably *Triticum turgidum* ssp. *dicoccoides*, exhibit substantial genetic variation for these nutritional traits, yielding zinc (Zn) concentrations spanning 14 to 190 mg kg^{-1} and iron (Fe) levels reaching 88 mg kg^{-1} (Gupta et al., 2024).

CIMMYT has been working with elite tetraploid *T. durum* or high Zn wild tetraploid wheat lines and the D genome progenitor *Aegilops tauschii* to create Synthetic Hexaploid Wheat (SHW) (Rosyara et al., 2019; Velu et al., 2019). Enabling over 1600 lines of SHW into a more comprehensive pipeline of breeding has significantly expanded the genetic diversity of micronutrient biofortification. The crosses of advanced lines of rye and various *Aegilops* species have been made to the adapted wheat background and have resulted in stable wheat genotypes with high levels of grain Zn (38–72 mg kg^{-1}) and Fe (32–52 mg kg^{-1}) for several years (Gupta et al., 2024).

This strong positive relationship in grain Fe and Zn concentrations consistently recorded ($r = 0.54$; $p<0.001$) enables both Fe and Zn to be enhanced concurrently through correlated selection (Gupta et al., 2024). This genetic link is important for Pakistan, where major population reliance on wheat.

Achievements in Pakistan

In collaboration with national agricultural institutions in Pakistan, India, Bangladesh and Bolivia, CIMMYT has released 40 biofortified wheat cultivars in the market. Currently five high zinc (Zn) varieties have been brought in for commercialization, Zincol-2016, Akbar-2019, and Nawab-2021 in Punjab Province and Tarnab Rehbar and Tarnab Gandum-I in Khyber Pakhtunkhwa (KPK) Province (Imtiaz, 2025).

The successful scaling up of this Akbar-2019 cultivar is a significant achievement in the biofortified crops dissemination. After commercialization in 2019, the area under cultivation of biofortified zinc (Zn) wheat increased to over 42% of the total wheat area in Pakistan. In the 2024-2025 agronomic cycle, the sown area of certified zinc wheat is estimated to be about 187 thousand of metric and is expected to produce more than 50% of the national wheat yield, approximately

15 million of mt of grain. This rapid agricultural integration as catalyzed by robust public private partnerships that mobilized over USD 85 million in annual investments. Around 80% of which comes from private seed companies, are more important (Mujahid, 2025). The parentage of these varieties shows the rational application of germplasm derived from CIMMYT. Zincol-2016 is derived from a complex cross of *T. spelta* accessions with elite bread wheat parents
(OASIS/SKAUZ//4BCN/3/2PASTOR/4/T.).SPELTA PI348449/5/BAV92/3/OASIS/SKAUZ// 4BCN/4/PASTOR/6/WBLL12/CHAPIO) and Akbar-2019 and Nawab-2021 are similar in their pedigrees with inclusion of high Zn synthetic wheat and landrace derivatives. Wheat is a self
pollinator and this self pollinating nature helps to maintain the genetic stability of these high-Zn traits over several generations of farmer's seed saving (Ram et al., 2024).

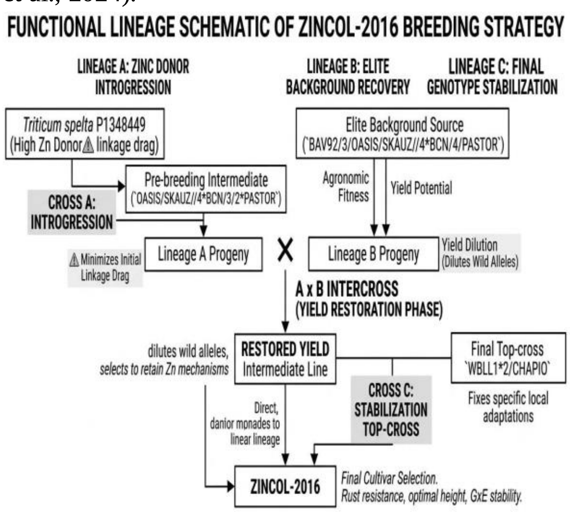

Figure 1: Breeding Pedigree of the zinc biofortified wheat cultivar Zincol-2016. The schematic illustrate the convergence of three primary lineages: the introgression of the high zinc donor Triticum spelta P1348449 (Lineagr A), the recovery of the eliteagronomic background (Lineage B), and the final genotype stabalizing utilizing highly adapted synthetic lines (Lineage C) to achive the commercial Zincol-2016 cultivar without yield penalties.

The Yield–Micronutrient Dilemma

A persistent challenge in conventional biofortification breeding is the negative correlation between grain yield and micronutrient concentration. Several studies have documented significant negative associations between grain yield and both Fe and Zn content, as well as between yield and phytic acid. This yield–nutrient trade-off is thought to arise from carbohydrate dilution effects, wherein increased carbohydrate accumulation during grain filling dilutes the concentration of minerals and proteins. In durum wheat, grain yield was negatively correlated with ZnC (r = -0.52) and FeC (r = -0.34) and phytic acid (r = -0.39) under reduced irrigation, suggesting that drought stress may exacerbate this antagonism (Magallanes-López et al., 2017).

For Pakistani breeders, this dilemma is particularly acute given the national imperative to increase per hectare wheat productivity to feed a growing population. The development of high yielding biofortified varieties therefore requires large population sizes, rigorous multi environment testing, and the strategic deployment of selection indices that weight both yield and micronutrient traits (Saini et al., 2020). Notably, the positive correlation between grain protein content and micronutrient concentrations offers an opportunity for indirect selection, as improving nutritional quality may concurrently enhance industrial baking quality (Magallanes-López et al., 2017)

Molecular Breeding Approaches

The quantitative nature of grain Zn and Fe concentration, controlled by multiple genes with small individual effects, makes conventional phenotypic selection inefficient and time consuming. Molecular breeding approaches including marker assisted selection (MAS), marker assisted recurrent selection (MARS), and genomic selection (GS) offer powerful tools to accelerate genetic gain by enabling selection at the DNA level (Sinha et al., 2023).

QTL Mapping and Marker-Assisted Selection

Numerous QTL mapping studies have identified chromosomal regions associated with grain Fe and Zn concentration in hexaploid wheat. A recent genetic dissection study identified 23 stably expressed QTLs across 21 wheat chromosomes, including four for grain Fe concentration (GFeC) and three for grain Zn concentration (GZnC). QTL clusters were identified where loci for GFeC co-occuring with those for thousand kernel weight (TKW), indicating that lines carrying favorable alleles at these loci can achieve higher grain Fe concentration and larger kernels simultaneously (Z. Zhang et al., 2026).

The identification of QTLs with stable expression across environments such as the major stable loci *QDH.cim-5B* and *QDH.cim-7D* with phenotypic variance explained (PVE), greater than 22% across environments provides molecular targets for selecting stability (Z. Zhang et al., 2026)

Targeted Trait	Chromosome Location	Specific QTL / Cluster	Key Impact & Significance
Grain Zinc Concentration (GZnC)	**7D**	QGZnC.cim-7D	A major stable locus specifically responsible for increasing zinc accumulation in the grain.
Yield / Seed Size (TKW)	**7B**	QTKW.cim-7B.2	A specific locus regulating Thousand Kernel Weight (TKW), essential for maintaining high yield.
Synergistic Clusters (Zn, Fe & Yield)	**2B, 5A, 7D**	**Multiple Pleiotropic Clusters**	These chromosomal "hotspots" control both micronutrient density (Zn/Fe) and yield traits simultaneously, breaking the traditional negative correlation between nutrition and yield.

Table 1: Major effect QTLs for controlling both nutritional quality and physical yield (Z. Zhang et al., 2026)

The pyramiding of favorable quantitative trait locus (QTL) alleles yields pronounced additive genetic effects. Among the tested QTLs, co-introgression of QTL for GFeC on chromosomes 2B, 5A and 7A in addition to QTLs for GZnC on 1B, 5B and 7D results in a much greater increase in micronutrient accumulation than single locus selection does (Z. Zhang et al., 2026). The use of marker-assisted selection (MAS) is a more efficient method than traditional backcrossing for incorporating these high value QTLs into elite, locally adapted germplasm and is particularly suited to Pakistan where complex regulatory constraints are a serious limitation on the rapid exchange of new varieties.

Effective implementation of MAS requires updated knowledge of the genetic basis of micronutrient homeostasis, including genes associated with efficient Fe and Zn uptake, translocation, and remobilization into grains. Several transporter families have been implicated in micronutrient trafficking, including ZIP (ZRT/IRT like protein) and IRT (iron regulated transporter) family members, as well as genes involved in the methionine cycle and phosphorus-sulfur biosynthesis pathways (Gupta et al., 2024).

Genomic Selection

Genomic selection (GS) predicts the genetic value of individuals using genomic estimated breeding values (GEBVs) derived from dense marker sets spanning the entire genome. Compared to MAS, which relies on a limited number of major effect QTLs, GS captures both major and minor genetic effects, making it particularly suitable for complex quantitative traits such as grain micronutrient concentration (Gupta et al., 2024).

In wheat, (Velu, Crossa, et al., 2016) reported genome wide prediction accuracies for grain Fe and Zn concentrations ranging from 0.331 to 0.694 and 0.324 to 0.734, respectively, in a diverse panel of 330 genotypes. These moderate to high prediction accuracies suggest that GS holds substantial potential for accelerating biofortification breeding. When combined with speed breeding protocols that enable up to six generations per year under controlled environments, GS can dramatically reduce breeding cycle duration and increase selection gain per unit time (Gupta et al., 2022).

For Pakistani breeding programs, the adoption of GS would require investment in high throughput genotyping infrastructure and the development of training populations representative of the country's diverse germplasm and environments. The integration of GS with multi environment trial data from Pakistan's distinct wheat zones irrigated Punjab, rainfed Potohar, and the warmer, water scarce regions of Sindh and Balochistan would be essential to account for genotype × environment (G × E) interactions that strongly influence micronutrient expression (Hayat et al., 2026).

Genome-Wide Association Studies and Candidate Gene Identification

Genome wide association studies (GWAS) have identified multiple marker trait associations (MTAs) for grain micronutrient content. A GWAS of phytic acid (PA) content in 330 CIMMYT breeding lines grown over three years revealed 21 genetic associations, six of which were consistent across years, mapping to chromosomes 1A, 2A, 2D, 5A, and 7D (Wen et al., 2022). Additivity across four of these haplotypes accounted for approximately 10% reduction in PA, demonstrating the feasibility of selecting for reduced anti nutrient content alongside elevated micronutrient concentration.

The nicotianamine synthase (NAS) gene family is one of the key targets for wheat biofortification. The gene family is made up of 21 identified wheat loci, which contribute to the complex genomic architecture of Fe homeostasis, acquisition and systemic translocation. *TaNAAT1*, *TaNAAT2*, and *TaDMAS1* are also induced in their roots but not in their shoots, while *TaNAS1* and *TaNAS2* are induced in both root and shoot tissues. The organized transcription activity boosts the absorption and the blood flow of Zn and Fe (Wani et al., 2022). Iron Deficiency-Responsive Elements (IDE1/IDE2) and Zinc Deficiency Responsive Element (ZDRE1 and ZDRE2) are cis regulatory elements, turns on in response to iron and zinc deficiency. These are promoter region for *TaNAAT* and *TaDMAS* genes. The expression of *TaNAAT* and *TaDMAS* upregulated during grain filling in flag leaves tissue and developing grains. It increase the level of 2-deoxymugineic acid (DMA) which act as a chelator for the phloem mediated translocation of iron and zinc directly to endosperm (Kumar et al., 2022).

Transgenic and Genome Editing Approaches

Although conventional and molecular breeding methods are effective, there is only a finite amount of genetic variation available in germplasm of sexually compatible cultivars that can be used to increase the micronutrient levels as high as possible. Biological constraints can be overcome by transgenic and genome editing technologies, which can be used to introduce new alleles or to target specific endogenous loci, maximizing the micronutrient content and bioavailability (Van Der Straeten et al., 2020).

Transgenic Approaches

Transgenic techniques have the added advantage that they can be adapted to several different species and may be used to introduce several key nutrients into a crop at the same time. In the rice crop, the overexpression of the gene *NICOTIANAMINE SYNTHASE2* (*OsNAS2*) in wheat significantly increased the concentration of grain iron (Fe) from 93.1 mg kg^{-1} under controlled greenhouse conditions to 80 mg kg^{-1} under field conditions (Gupta et al., 2024). From a mechanistic standpoint this enrichment of NA is attributed to the synthesis of NA mediated by NAS. NA is a non proteinogenic amino acid, it plays an important role in plant internal mobilization and distribution of micronutrients, especially Fe and zinc (Zn) in the whole plant structure, in its chelated form (Cortelezzi et al., 2025).

The *FERRITIN* gene has been identified as a candidate for biofortification in crops due to its unique ability to bind up to 4500 iron (Fe) atoms. The resulting transgenic plant has shown very divergent results with only the over expression of *FERRITIN*. The Fe uptake and vascular translocation networks need to be optimized at the same time. Not only by increasing the elemental sink is enough. Optimizing all these at once has been complicated. In wheat, the coexpression of NAS and *FERRITIN* failed to increase Fe accumulation beyond the levels achieved by single gene interventions. This absence of an additive effect exposes complex, species-specific metabolic bottlenecks that must be resolved to advance transgenic enrichment strategies (Cortelezzi et al., 2025). Vacuolar iron transport has also been targeted. (Connorton et al., 2017) demonstrated that the use of the *VACUOLAR IRON TRANSPORTER2* (*TaVIT2*) gene effectively doubled the total Fe content in wheat flour by enhancing vacuolar Fe and manganese transport in the endosperm. Iron transporters are not highly expressed in the starchy endosperm (white flour) tissue. Baseline expression of iron transporters is very low in the starchy endosperm (white flour) tissue. This means that the iron-rich bran and germ are removed during milling. The coding sequence for TaVIT2 is inserted into the genome with an endosperm specific promoter (Glu-1Dx5). Iron is supplied to the developing grain through the phloem and must cross the endosperm to enter the embryo.

To increase Fe bioavailability, phytic acid content was decreased by silencing the wheat *TaABCC13* transporter gene. RNAi is the most widely used technique to knock down *TaABCC13* in the literature. The design of an RNAi construct comprises a promoter (constitutive such as Ubi or seed specific) that is followed by inverted repeats of a highly conserved region of *TaABCC13*. This is transcribed to form a hairpin RNA (hpRNA). The hpRNA is cleaved by endogenous Dicer-like enzymes within the plant to generate small interfering RNAs (siRNAs) that deliver the RNA induced silencing complex (RISC) to target the endogenous mRNA of all three *TaABCC13* homoeologs (Bhati et al., 2016).

Despite these promising proof-of-concept results, transgenic wheat biofortification remains limited in practical application. The scanty work in traditional transgene based approaches for wheat may be associated with issues of transgene stability across generations, the logistical demands of large scale field trials, and most importantly the restrictive regulatory policies governing transgenic plants globally (Gupta et al., 2024). Regulatory hesitation is now one of the main constraints that hinders the agricultural translation and wider roll out of transgenic biofortified wheat.

Genome Editing via CRISPR/Cas9

The introduction of genome editing using the CRISPR/Cas9 system has revolutionized the concept of crop improvement by allowing to make specific and targeted changes in the genome without adding foreign DNA. This approach avoids the addition of foreign genes and is viewed as having a better safety record than traditional transgenic methods, making it more likely to be accepted by the public and regulators globally. CRISPR engineering is an extremely translational and practical method to improve the availability of micronutrients in staple food crops.

The wheat reference genome is publicly available and the wheat genome assemblies (IWGSC RefSeq v1.0 and later) have provided the wheat genome resources to design highly specific guide RNAs for targeted editing of genes controlling micronutrient homeostasis (The International Wheat Genome Sequencing Consortium (IWGSC) et al., 2018).

In wheat, CRISPR/Cas9 has been successfully used to edit several traits. It includes decreasing the amount of gluten through the editing of alphagliadin genes and increasing Fe and Mg content by targeting *TaVIT2* (Bhattacharya et al., 2024). CRISPR/Cas9 editing can be used for a full knockout of *TaABCC13*. Single guide RNAs (sgRNAs) are designed against highlyconserved exons (usually that of critical nucleotide binding domains (NBDs) or transmembrane domains (TMDs)) across the 4B, 4D and 5A subgenomes. This causes the simultaneous occurrence of double strand breaks, which leads to frameshift mutations and therefore inactivity of the transporter (Ibrahim et al., 2022).

CRISPR biofortification has numerous strategic benefits for Pakistan. The technology can be used to knock out or downregulate genes involved in phytic acid biosynthesis such as inositol phosphate kinase genes (Sparvoli & Cominelli, 2015). Modify promoter regions of metal transporter genes to enhance expression Fe accumulation (Masuda et al., 2012) under Fe deficient soil conditions prevalent in Pakistan. The application of CRISPR in wheat biofortification is still in its infancy, and significant technical challenges remain, including the hexaploid nature of wheat (which complicates homozygous editing across all three sub genomes) (Matres et al., 2021), low transformation efficiency, and offtarget effects (Angon et al., 2026).

Challenges and Opportunities in the Pakistani Agricultural Sector

Edaphic Constraints and Micronutrient Bioavailability

The biofortification potential of any wheat variety is fundamentally constrained by the soil environment in which it is grown. Pakistan's wheat belt is dominated by calcareous, alkaline soils derived from Indus alluvium, characterized by high pH (7.8-8.5), low organic matter

The biofortification potential of any wheat variety is fundamentally constrained by the soil environment in which it is grown. Pakistan's wheat belt is dominated by calcareous, alkaline soils derived from Indus alluvium, characterized by high pH (7.8-8.5), low organic matter (<1%), and elevated calcium carbonate content (Rehman et al., 2020). These conditions promote the precipitation of Zn as $Zn(OH)_2$ and $ZnCO_3$ and its adsorption onto clay and carbonate surfaces, rendering a large proportion of soil Zn unavailable for root uptake (Khan et al., 2023).

In Pakistan, field evaluations confirm the agronomic potential of direct nutrient supplementation with soil applied zinc (Zn) which can significantly boost wheat yields by 18 to 41% over non supplemented control in alkaline soils (Khan et al., 2023; Rehman et al., 2020). The wheat production improvements are considerable, the effectiveness of this traditional method is fundamentally limited by the local geochemistry. Fast chemical fixation reactions in alkaline soil matrix result in the immobilisation of the applied Zn, with an inherent reduction in its solubility and thus bioavailability to the crop (Khan et al., 2023). In the Potohar plateau, rainfed wheat was found to be Zn deficient in more than 80% of sampled fields, with a fertilizer requirement of 2.0 kg Zn ha^{-1} for near maximum grain yield (Rafique et al., 2006). Varieties must possess enhanced Fe chelating with phytosiderophores (X. Zhang et al., 2019) to access sparingly soluble Zn and Fe fractions. The genetic potential for high grain Zn expressed under optimal soil conditions may not be fully realized under Pakistani field conditions, necessitating that selection for biofortification traits be conducted directly in target environments with representative soil constraints.

Genotype × Environment Interactions and Climate Resilience

Micronutrient accumulation in wheat grain is highly sensitive to environmental variation, and G × E interactions represent a major challenge for stable biofortification. Heat stress, in particular, has been shown to reduce both Zn and Fe concentrations in wheat grain. (Panigrahi et al., 2022) reported reductions of approximately 32% for Fe and 6% for Zn under heat stress conditions. With projected temperature increases in South Asia, these declines threaten to undermine the nutritional gains achieved through breeding.

In Pakistan, wheat is cultivated across highly heterogeneous environments. The irrigated plains of Punjab and Sindh experience high temperatures during grain filling (March-May), while the rainfed Potohar plateau faces terminal drought stress (Sattar et al., 2020). The negative correlation between grain yield and micronutrient concentration is exacerbated under water deficit, as observed in durum wheat where reduced irrigation significantly lowered Zn concentration despite higher Fe content (Magallanes-López et al., 2017).

The Varietal Release and Seed System Bottleneck

Highly complex and multi tiered regulatory framework is currently impeding commercialisation of new crop varieties in Pakistan. The process from genetic crossing to market release takes 6 to 8 years as it is guided by federal and provincial obligations set by the Seed Act of 1976 and its 2015 amendment. This long pathway requires preliminary and advanced yield trials both regionally and nationally, with the result being the final approval by Provincial Seed Councils headed by Ministers (Imtiaz, 2025).

In the case of biofortified cultivars, this long developmental period is even longer due to the need to conduct specific tests to demonstrate micronutrient levels (Imtiaz, 2025). Pakistan’s agricultural sector relies predominantly on uncertified, farm saved seed for routine wheat cultivation, the rapid and widespread dissemination of high value, certified biofortified seed remains profoundly restricted (Hussain et al., 2017).

Bioavailability Constraints: The Phytic Acid Challenge

Increasing the levels of zinc and iron in grains is a critical first step toward reducing micronutrient deficiencies, but it is fundamentally constrained by the bioavailability of these micronutrients when humans digest their food. Main obstacle is the phytic acid (myo-inositol hexakisphosphate) found abundantly in the bran and aleurone layers of wheat. Phytic acid is a powerful chelator of divalent cations such as Zn^{2+} and Fe^{2+}, which is because the human small intestine does not contain the enzyme phytase to hydrolyse it. This interaction results in the formation of insoluble complexes that do not get absorbed into the intestine and eventually block physiological absorption of these essential elements (Brouns, 2021).

The molar ratio of phytic acid (PA) to Fe and Zn is established stoichiometric proxy for Fe and Zn bioavailability and used to predict physiological uptake. The ratios determine relative expected absorption efficiencies, with high (approximately 50%), moderate (which is about 30%), and low (approximately 15%) levels. The PA:Fe ratio ranging from 12.1 to 29.6 and the PA:Zn ratio between 16.9 and 23.6, observed in commercial cultivars of durum wheat when

compared to these benchmarks, reveal a very consistent and limited micronutrient availability (Magallanes-López et al., 2017). Biochemical barrier exists even in advanced biofortifications programmes. The total PA content in the breeding population of the HarvestPlus program spans from 0.90% to 1.72% while several new varieties with high PA content are still not reaching the optimal PA:Zn or PA:Fe molar ratio required to demonstrate the effect on the mineral content of the grain and the resulting nutritional benefit (Wen et al., 2022).

Future Outlook and Strategic Recommendations

Integrating Multi-Pronged Breeding Strategies

Conventional breeding must continue to be the core strategy to this national initiative and more focus must be placed on CIMMYT's high zinc germplasm, as well as synthetic hexaploid lines (Velu et al., 2022). This dependence is based on the fact that this method is proven to be efficient, economical and is accepted by agricultural communities (Lowe et al., 2020). Combining marker assisted selection (MAS) for high trait heritability quantitative trait loci (QTLs), with the traditional foundation can significantly accelerate the introgression of improved micronutrient gene parts into locally adapted cultivars (Doktyrbay et al., 2026; Nasim et al., 2025). Selection of the traits with consistent phenotypic expression in the different agroecological zones across Pakistan will be important for achieving rapid, resilient and context specific nutrition improvement (Ahmad et al., 2024).

Systematic implementation of Genomic Selection (GS) is a formidable opportunity to improve our ability to biofortify crops as the costs of high throughput genotyping continue to drop (Gupta et al., 2022). Development of a national GS infrastructure, with reference genome as a central platform and resequence data from locally adapted germplasm from Pakistan, would help to alter the breeding paradigm from empirical to the concept of predictive modeling. This structure would be efficient to single out the complex micronutrient traits and would bypass the logistical issue of an extensive, generation by generation phenotyping process. Genomic Selection (GS) combined with higher levels of speed breeding can significantly shorten the cultivar development process (Voss-Fels et al., 2019). This approach is quick and efficient in bringing nutrient rich options to the market.

High Throughput Phenotyping and Multi Environment Testing

he analytical bottlenecks in scaling up the biofortification program is vital, and it depends on the use of reliable and efficient platforms for high throughput phenotyping of grain zinc, iron and phytic acid. The use of X-ray fluorescence (XRF) spectroscopy is particularly important because it is a non destructive diagnostic method that has enabled thousands of breeding lines to be evaluated on an annual basis (Chakraborty & Shrivastav, 2024). There is also a need for high throughput capabilities to monitor antinutritional factors in a parallel fashion. A 96 well megatiter plate assay, using a commercially available phytase, has proven to be highly scalable and to facilitate rapid and direct identification of desirable phytic acid profile in existing forward breeding pipelines (Wen et al., 2022). Stable phenotyping of improved nutrition traits will need systematic incorporation of tests in multiple environments in the different wheat production zones of Pakistan. Regional evaluations are an important precondition for breeding success, because the micronutrients accumulate in the plants very sensitive on the complex and localized environmental variables Specialized biofortification screening nurseries need to be built in representative agroecological zones to reflect these genotypes by environment (G × E) interactions accurately. (Imtiaz, 2025; Mahmood et al., 2024) published the Pakistan's regional capabilities for national trials of elite varieties at different locations.

Developing Climate Resilient Biofortified Varieties

The nutrition improvements obtained by crop biofortification in Pakistan can still be compromised by climate change. Rising temperatures and droughts (Velu, Guzman, et al., 2016), combined with increased atmospheric CO_2 (Maqbool et al., 2020), will both be expected to reduce the stability of crop yields and lower concentrations of zinc and iron in the grain, at the time of grain set and grain filling, which is when these micronutrients are most important to crops. It is crucial that future agricultural breeding programs begin to purposefully focus on integrating micronutrient biofortification targets with also to adapt to climate induced stress traits like optimal water use efficiency, as well as heat and drought tolerance, on a systematic basis, in order to continue these nutritional benefits (Elbasyoni et al., 2026).

Improving both abiotic stress tolerance and accumulation of micronutrients in agriculture will demand a deliberately focussed approach to CRISPR Cas gene editing, and molecular marker assisted selection (MAS). The interrelationships between these traits have a physiological dimension with actionable synergies for improvement of crops (Saha et al., 2025). The negative relationship between days to heading and accumulation of Zinc (Zn) represents a crucial strategic advantage (Thapa et al., 2022). Use of early maturing cultivars is a good way to both avoid terminal heat stress and maximize nutrient loads during the grain filling phase. The positive correlations between thousand kernel weight and Zn density highlight an intriguing linkage between breeding traits and nutritional security, suggesting that achieving yield improvements could also contribute to improved mineral nutrition (Z. Zhang et al., 2026).

Strengthening the Seed Value Chain

A successful scaled up of Akbar-2019 cultivar demonstrates the feasibility of intensive deployment of biofortified crops when private companies, government research institute and government extension networks work together (Ahmad et al., 2024; Bouis & Saltzman, 2017). The current situation, characterized by the dominance of public sector production and the weak involvement of the private sector in certified seed production, represents an alarming supply constraint. This structural restriction hinders availability of good quality biofortified seed, which disproportionately affects the marginalised farming communities in geographical isolation, and resource poor areas (Abedullah & Ali, 2024).

Consumer Acceptance and Nutrition Education

Effective transition of agronomic biofortification to real life public health outcomes greatly relies on consumer acceptability and dietary incorporation. The subtle wheat coloration associated with high zinc varieties, a change which relates to a higher concentration of pigments in the outer aleurone cell layers, may create a challenge to uptake in Pakistan where wheat is mainly consumed as the whole grain chapati (Ortiz-Monasterio et al., 2007). For market viability, the breeding schemes should be designed to maximise visual conformation to cultural preferences. HarvestPlus have helped to launch the first waves of awareness programmes. These frameworks also need to explicitly state the physiological need for biofortification, with awareness of its importance for early childhood development, maternal health, and immune function (Ohly et al., 2019).

Conclusion

Breeding for zinc (Zn) and iron (Fe) biofortification is a critical intervention in a country like Pakistan where wheat is the base of agriculture and food consumption. Traditional agronomic practices using CIMMYT germplasm, wild crop species and synthetic hexaploids have proven to be effective for the generation of marketable cultivars (e.g., Zincol-2016 and Akbar-2019) (Wani et al., 2022). These efforts results in the cultivation of these varieties for about 42% of the national wheat area (Ahmad et al., 2024). The urgent integration of cutting edge molecular and biotechnological platforms into the existing crop improvement processes will provide a better deployment of biofortification breeding program.

Marker assisted and genomic selection are important advanced molecular breeding methods to enable the process of pyramiding alleles that lead to zinc and iron enrichment while maintaining yield and climate adaptability (Wani et al., 2022). CRISPR/Cas9 genome editing is a game changing strategy for addressing micronutrient bioavailability constraints that extend beyond mere selection. This cutting edge technology has the potential for the lowering of crop phytic acid content and alteration of expression of metal transporters (Cominelli et al., 2020). Pakistan's crop nutritional integrity is facing serious threat of climatic volatility and strong edaphic constraints such as calcareous profiles and high soil alkalinity, making the need for integration of these biotechnological tools an all the more special and pressing need of Pakistan. Achieving long term nutritional security in this area requires the development focused on root yields from crops with capacities to express efficient levels and steady tonnes of micronutrients across the variability of the environment (Rehman et al., 2018).

The scientific progress in conjunction with adequate institutional collaboration and adaptable policy frameworks offers a pragmatic pathway to lift wheat production from secondary status to be a major contributor to national nutrition security. But implementing this framework requires sustained investment programmes throughout the developmental pipeline. Some of the most important tasks will involve further upgrading of breeding facilities, high throughput phenotyping, multi environment testing and an extensive upgrade of seed supply chains and regulatory procedures.

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