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BIOFORTIFICATION THROUGH MODERN BREEDING: GENETIC AND GENOMIC STRATEGIES FOR NUTRITIONAL SECURITY

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ABSTRACT

Even though countries like India and China has achieved the sustainable food production for meeting the food requirements of their respective countries, still some section of people are suffering from micronutrient malnutrition or hidden hunger due to micro nutrient deficiency, often of iron, zinc, folate or vitamin A. The staple cereals, legumes, and root crops are bulk in dietary energy yet are poor sources of several micronutrients. To increase the nutrient density in the food crops, modern breeding techniques are introduced to complement supplementation and industrial fortification. This review discusses about the genetics and genomics of crop biofortification from classical quantitative trait locus (QTL) mapping and marker-assisted selection through genome-wide association studies (GWAS), genomic selection and CRISPR-Cas genome editing. It examines the molecular architecture of mineral homeostasis (ZIP, NRAMP, YSL, VIT, ferritin, and nicotianamine synthase gene families), Provitamin A carotenoid pathway (crtRB1 and IcyE), and endosperm protein quality loci (opaque 2, opaque 16). It also discusses about the emerging integration of genomic selection with speed breeding and multi-omics/pan-genome resources. While the single nutrient biofortification is now a scalable technique for several staple crops, the merging techniques focus on stacking multiple micronutrient and quality traits within high-yielding, climate-resilient through precision genomics.

KEYWORDS: Biofortification, QTL mapping, GWAS, CRISPR-Cas, Genomic selection, Marker-assisted selection.

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INTRODUCTION

Food security is not merely dependent on quantity of food consumed but ensuring access to safe, nutritious, and diverse food that meet both the dietary and micronutrient requirements. Adequate nutrition provides essential macronutrients such as carbohydrates, proteins, and fats along with micronutrients containing vitamins and minerals that are necessary for optimal growth and development. Global food systems have been persistently

underperformed in producing food with micronutrient quality. Even though calorific availability has risen over the past decade, deficiencies of zinc, iron, folate, vitamin A, and iodine remain widespread. This is widely known as “hidden hunger” due to its subclinical symptoms yet cumulatively severe, ranging from impaired cognitive development and anemia to increased susceptibility to infections and maternal mortality (Muthayya et al., 2013; Yilmaz and Yilmaz, 2025). Population representative survey by the Lancet Global Health estimated that one in two pre-school aged children and two in three reproductive age women have at least one micronutrient deficiency worldwide (Stevens et al., 2022; Micronutrient Forum /GAIN, 2022). This is particularly pronounced in South Asia and sub-Saharan Africa, where diets majorly rely on a limited range of staple cereals and root crops, it remains a concern even in high income countries.

Malnutrition is a major global challenge amid persistent population growth. The world population is projected to increase to approximately 8.6 billion by 20230, raising pressure on food systems to provide sufficient and nutritionally quality food. According to the Food and Agriculture Organization (FAO), almost 673 million people, representing 8.2% of the global population, experienced hunger/undernourishment in 2024, highlighting the persistent challenge of global food and nutritional security. Most people in developing countries either face hunger or receive nutrient-poor food. Micronutrient deficiencies, commonly called hidden hunger, affect more than two billion people worldwide, representing approximately one-third of the global population, and remain a public health challenge, majorly in low and middle income countries (Chaudhary et al., 2025). They lack either one or all of the essential micro-or micronutrients like Zn, Fe, selenium, iodine, folic acid, lysine, vitamin A, vitamin B12, vitamin C, vitamin D, and others in their diet. These vitamins and nutrients are inevitable for the growth, development, and proper functioning of the human body (FAO, 2002). Prolonged inadequate intake of essential micronutrients can result in significant deficiency disorders like anemia (Fe deficiency), beriberi (Vitamin B deficiency), pellagra (niacin deficiency), rickets (vitamin D deficiency), and scurvy (vitamin C deficiency) or be fatal. Most people depend on cereals and pulses to fulfill their dietary requirements (WHO, 2025). Cereals are a major component of the food supply, accounting for approximately 42% of dietary energy supply worldwide in 2023, while pulses also contribute to food and protein supplies (FAO, 2025). However, cereal does not have a balanced nutritional profile, i.e., iron and zinc and other essential nutrients, which is the leading cause of malnutrition.

Three complementary strategies to address the micronutrient malnutrition at population scale; dietary diversification, industrial fortification of processed foods, and biofortification of staple crops. Biofortification is the enhancement of nutritional quality of food crops through agronomic practices, breeding or biotechnological strategies, is different from fortification for its recurring, low-marginal cost delivery. Once a nutrient dense variety is developed and its seed enters the supply chain, the intervention can be sustained across subsequent growing seasons without requiring repeated point-of-sale investment (Bouis and Saltzman, 2017; De Steur et al., 2022). Agronomic biofortification through soil or foliar application of micronutrients can enhance mineral concentrations in grain; however its effectiveness is constrained by input costs, soil chemistry, and recurrent applications. Instead the genetic biofortification incorporates micronutrient rich traits into the seed, making it a sustainable strategy for long-term nutritional improvement (Bouis and Saltzman, 2017; Kumar and Rani, 2024). The genetic potential of biofortification is supported by three empirical evidences accumulated over the past two decades: firstly, heritable variation for grain mineral density, Provitamin A, essential amino acid content within crop germplasm collections and crop wild relatives; second the variation can be mapped to specific chromosomal regions and to specific candidate genes that control nutrient uptake, transport and storage; thirdly favorable alleles can be introgressed into elite varieties (Garcia-Oliveira et al., 2018; Sharma et al., 2021).

This review is centered on genetics and genomics through marker assisted selection, genome wide association mapping, genomic selection, and CRISPR-Cas genome editing approaches for biofortification. (Fig. 1).

2. Genetic Architecture underlying Target Nutrient Traits

Most traits of nutritional significance that biofortification breeding aims to enhance, such as the density of iron and zinc in grains, the accumulation of provitamin A carotenoids, folate levels, and the composition of essential amino acids, are quantitative traits influenced by numerous genes with small to moderate effects. These traits are significantly affected by genotype-by-environment interactions. Advances in understanding this genetic architecture have largely relied on the availability of high-quality reference genomes, comprehensive SNP genotyping platforms, and well-structured mapping populations. These resources have seen rapid expansion across major staple crops.

2.1 Iron and Zinc Homeostasis Genes

Iron and zinc accumulation in grains relies on a well-preserved sequence involving soil uptake, xylem transport from roots to shoots, storage in vegetative tissues, phloem remobilization during senescence, and eventual deposition into the endosperm or aleurone (Fig.2). Gene families frequently associated with this process in cereals and legumes include the ZIP (ZRT/IRT-like protein) and NRAMP (natural resistance-associated macrophage protein) influx transporters, the YSL (yellow stripe-like) and MATE-family transporters that facilitate the movement of metal-nicotianamine complexes through the phloem, the VIT (vacuolar iron transporter) and MTP (metal tolerance protein) families responsible for vacuolar sequestration, ferritin genes for storage, and the nicotianamine synthase (NAS) and nicotianamine amino transferase (NAAT) genes that produce metal chelators for long-distance transport (Rathan et al., 2021; Rashid et al., 2025).

In rice, quantitative trait locus (QTL) mapping conducted across biparental, backcross, and multi-parent

advanced generation intercross (MAGIC) populations has observed over ninety loci linked to grain iron content and a similarly extensive number for zinc, with recurring hotspots located on chromosomes 1, 3, and 7. Candidate genes in these regions include OsNAS1, OsNAS2, OsNAS3, OsVIT1, OsVIT2, OsNRAMP1, OsNRAMP7, OsMTP1, OsMTP6, and OsHMA1, several of which have been functionally validated through knockout or overexpression experiments (Sanjeeva rao et al., 2020; Descalsota et al., 2018). In wheat, the identification of the Gpc-B1 locus and its associated gene, the NAC-family transcription factor NAM-B1, was a pivotal discovery. The functional allele of this gene speeds up leaf senescence and nutrient remobilization, leading to an approximate 18% rise in grain iron and a 12% increase in grain zinc compared to the non-functional allele (Uauy et al., 2006; Distelfeld et al., 2007, Velu et al., 2020). Following the 2018 release of the IWGSC Ref Seq v1.0 chromosome-scale wheat genome, genome-wide association studies using the HarvestPlus wheat association-mapping panel and related diversity panels have pinpointed numerous additional loci linked to zinc and iron, including significant regions on chromosomes 2 and 7. More recently, twenty-five and sixteen stable loci for grain zinc and iron, respectively, have been identified using 660K and 90K SNP arrays and confirmed with Kompetitive allele-specific PCR (KASP) markers.

In pearl millet, quantitative genetic research has consistently shown high broad-sense heritability for grain iron (approximately 0.94) and zinc (around 0.90), with mainly additive gene action. This combination supports straightforward phenotypic and marker-assisted selection over more intricate heterotic breeding strategies (Rai et al., 2012). Diagnostic SNP markers for high-iron haplotypes and co-located drought-tolerance loci have been integrated into rapid early-generation screening processes at ICRISAT (Govindaraj et al., 2024). In common bean, meta-QTL analyses spanning multiple biparental and association-mapping studies have converged on chromosome Pv06 as a consistent hotspot for seed zinc concentration, alongside PvZIP17 and PvZIP19 as candidate loading genes and PvMTP4, PvMTP5, and PvMTP12 as candidate vacuolar transporters (Izquierdo et al., 2018).

2.2 Provitamin-A Carotenoid Pathway Genes

Provitamin A biofortification aims to enhance the concentration of β-carotene and similar carotenoids in consumable parts of plants. In maize, natural allelic variations at two key genes in the carotenoid biosynthesis pathway, crtRB1 (β-carotene hydroxylase 1) and lcyE (lycopene-ε-cyclase), shift the metabolic process towards β-carotene instead of leading to xanthophylls or the α-branch of the pathway. Favorable crtRB1 alleles alone can significantly increase the provitamin A content in kernels, surpassing the typical yellow maize levels, which usually have less than 2 micrograms per gram, to a recommended breeding target of approximately 15 micrograms per gram (Zunjare et al., 2018). Since these alleles segregate naturally and are not transgenes, they have been widely utilized through marker-assisted backcrossing. In contrast, rice starts from a different genetic baseline: the carotenoid biosynthesis pathway is inactive in the endosperm of all traditional varieties, making it impossible to revive provitamin A production through allele mining within the species. Golden Rice is developed through the transgenic insertion of two specific genes: a plant phytoene synthase, initially sourced from daffodil and later substituted with a maize psy1 orthologue in the GR2E second-generation event, and a bacterial carotene desaturase (crtI, originally from Erwinia uredovora and subsequently from Pantoea ananatis). These genes together reconstruct the pathway from geranylgeranyl diphosphate to lycopene, eventually leading to β-carotene production. This results in rice grains that, in the second-generation event, accumulate approximately 20 to 30 micrograms of β-carotene per gram of milled rice (Palmer, 2025; Dubock et al., 2021).

In the case of cassava, the BioCassava Plus programme adopted a similar transgenic approach by expressing a deregulated version of phytoene synthase to boost carotenoid production in storage roots. This not only increased provitamin A levels and extended post-harvest shelf life but also affected the distribution of carbon between starch and carotenoid biosynthesis, impacting the dry-matter content of storage roots and necessitating further breeding improvements (Beyene et al., 2018). Similar carotenoid pathway engineering approaches, such as the introduction of the *Orange (Or)* gene variant identified in cauliflower, have been explored across potato, banana, and other vegetatively propagated crops, where conventional recombination is constrained by limited natural genetic variation (Li et al., 2001).

2.3 Genetic Loci underlying Protein Quality and Nutrient Density

Quality protein maize (QPM) stands as an early and lasting achievement in genetic biofortification, originating before the genomics era but now seamlessly incorporated into marker-assisted breeding processes. The recessive opaque2 (o2) mutation, located on chromosome 7 and coding for a bZIP-family transcription factor, approximately doubles the lysine and tryptophan content in the endosperm by inhibiting the production of lysine-deficient zein storage proteins. However, the initial o2 mutants had soft, chalky kernels that were susceptible to pest damage and yield reduction. QPM breeding addressed this issue by utilizing endosperm "modifier" loci, which are associated with the expression of 27-kilodalton γ-zein, to restore a hard kernel phenotype while maintaining the amino acid advantage (Hossain et al., 2019). Additionally, a second recessive locus, opaque16 (o16) on chromosome 8, increases lysine by 30 to 40% and tryptophan by 50 to 60% when combined with o2, without causing kernel opacity, making it a valuable complementary target for marker-assisted gene stacking (Sarika et al., 2018). A notable aspect of recent maize biofortification breeding has been the concurrent marker-assisted integration of crtRB1, lcyE, and o2 (and in some cases, o16) into single elite hybrid backgrounds. This approach merges the enhancement of provitamin A with improvements in protein quality within one product. Through foreground selection using gene-based SSR and InDel markers across successive backcross generations, recovery of the recurrent-parent genome has been achieved between 88% and 96%. The resulting hybrids exhibit

several-fold increases in provitamin A and 1.5- to 2-fold increases in lysine and tryptophan compared to their unimproved counterparts (Zunjare et al., 2018; Mehta et al., 2020).
In addition to minerals, provitamin A, and amino acids, breeding programmes have also focused on reducing antinutrients that hinder mineral bioavailability. This includes targeting phytic acid reduction through low-phytate (lpa) alleles in cereals and legumes, and specifically in legume crops, decreasing tannin content to enhance iron and protein bioavailability (Kumar et al., 2022). Folate biofortification, targeting genes in the GTP cyclo hydrolase I and amino deoxychorismate synthase branches of the folate biosynthetic pathway, remains comparatively early-stage in conventional breeding and has so far been pursued predominantly through metabolic engineering and, more recently, CRISPR-based cis-regulatory editing strategies (Khan et al., 2024). (Fig. 2).

3. Genomics Assisted Breeding Strategies

The translation of mapped QTLs and candidate genes into deployable varieties has depended on a sequence of increasingly sophisticated genomics-assisted breeding (GAB) tools, each addressing, specific bottlenecks in the classical pedigree-breeding cycle.

3.1 Marker Assisted Selection and Backcrossing

Marker-assisted selection (MAS) and marker-assisted backcrossing (MABC) continue to be the primary techniques for incorporating single major-effect alleles, such as crtRB1, o2, o16, or Gpc-B1/NAM-B1, from a donor into a recurrent parent that is already adapted. This is achieved while employing background markers to speed up the recovery of the recurrent parent's genome. This strategy has been repeatedly and effectively applied in maize, focusing on crtRB1, lcyE, o2, and o16, as well as in wheat, where NAM-B1 functional alleles have been introduced, and more recently, KASP-validated zinc QTL on chromosomes 3A and 7A have been incorporated (Tong et al., 2022; Owens et al., 2014; Zunjare et al., 2018). Marker-assisted recurrent selection (MARS) and haplotype-based breeding build on this approach by targeting traits governed by multiple loci with moderate effects, enabling the concurrent enhancement of several QTL across breeding cycles instead of focusing on a single locus at a time (Sinha et al., 2021).

3.2 Genome-Wide Association Studies

Genome-wide association studies (GWAS) utilize historical recombination events within diverse germplasm collections to achieve much finer mapping resolution compared to biparental QTL analyses. However, this approach necessitates larger, well-defined association panels and meticulous management of population structure. The emergence of chromosome-scale reference genomes, notably the 2018 IWGSC RefSeqv1.0 assembly for bread wheat, along with high-density SNP genotyping arrays (such as the wheat 660K and 90K arrays, and their rice and maize counterparts), has significantly boosted the identification of micronutrient-associated loci through GWAS since 2018 (Ali and Borrill, 2020; Tong et al., 2022; Owens et al., 2014). In rice, a GWAS conducted on a 144-line multi-parent advanced generation intercross (MAGIC) population pinpointed seven loci linked to grain iron and zinc content, with one locus on chromosome 7 (qZn7.1) demonstrating consistent stability across four different environments (Descalsota et al., 2018). Similar studies using panel-based approaches in wheat have uncovered thirty-nine marker-trait associations for zinc content, with significant loci located on chromosomes 2 and 7, utilizing the HarvestPlus association-mapping panel (Velu et al., 2018).

3.3 Genomic Selection and Genomic Prediction

Genomic selection (GS) moves away from tracking individual locus markers and instead employs genome-wide marker data to develop statistical or machine-learning models. These models predict genomic estimated breeding values (GEBVs) for each member of a breeding population, even for those not directly phenotyped for the desired trait. For traits that are quantitative, have low heritability, or are costly to phenotype—such as grain mineral density, which is expensive to measure using atomic absorption spectroscopy or ICP-MS assays on a large scale—GS significantly enhances selection accuracy and reduces cycle time compared to phenotypic or single-marker selection methods (Fels et al., 2019; Vasistha & Sheikh, 2026). Editorial and review analyses of literature from 2019 to 2024 highlight that the main challenges in implementing GS for biofortification traits are the high genotyping costs for smaller public breeding programs and the potential loss of genetic diversity for traits like disease resistance, which are not directly included in the prediction model due to intensive selection on a limited set of markers (Mishra et al., 2023; Vasistha & Sheikh, 2026).

3.4 Synergistic Integration of Speed Breeding and High-Throughput Phenotyping

A notable methodological advancement in the 2020s has been the systematic merging of genomic selection with speed breeding. This involves using controlled-environment techniques that extend photoperiods and adjust temperatures to reduce generation times in long-day cereals and legumes. The integration of genomic selection-based parent selection with accelerated generation cycles, often referred to as "Speed GS," has been both theoretically modeled and practically tested. This approach aims to optimize genetic gains per unit time for traits like nutrient density, which would typically necessitate several seasons of field phenotyping per cycle (Ceran et al., 2024; Watson et al., 2019; Bohra et al., 2021). Concurrently, the development of high-throughput phenotyping platforms, including hyperspectral imaging, portable X-ray fluorescence (XRF) spectrometry, and near-infrared spectroscopy calibrated against reference wet-chemistry mineral assays, has significantly lowered costs and increased the efficiency of grain mineral phenotyping. This progress has made it feasible to implement genomic selection training populations on a breeding program scale (Sinha et al., 2021; Sandhu et al., 2022).

(Table 1)

4. Crop Specific Case-Studies

The following case studies illustrate how the genetic and genomic tools described above have been sequenced and combined within individual crop breeding programmes, several of which have produced varieties now grown widely by farmers.

4.1 Rice Biofortification: Fe/Zn QTLs to Golden Rice

Rice biofortification has advanced along two main paths that have remained mostly distinct. The mineral-density approach has utilized traditional QTL pyramiding and, more recently, genomic selection within elite indica and japonica varieties, taking advantage of the extensive list of Fe- and Zn-related QTL and candidate genes detailed in Section 2.1. Lines with gene pyramiding that combine beneficial OsNAS, OsVIT, and OsNRAMP alleles with high-yield backgrounds have been developed through several national breeding programs, including those in India and the Philippines, coordinated by HarvestPlus and IRRI (Senguttuvel et al., 2023). On the other hand, the provitamin A approach has relied on transgenesis through Golden Rice, as no natural rice germplasm expresses the carotenoid pathway in the endosperm. After an approximately twenty-year regulatory process, the Philippines approved the commercial cultivation of the second-generation GR2E Golden Rice event in July 2021, with Bangladesh soon following, and food-use approvals already in place in the United States, Canada, Australia, and New Zealand (De Steur et al., 2022; Palmer et al., 2025).Since these approvals, research has focused on integrating the GR2E trait into locally preferred, higher-yielding varietal backgrounds through backcrossing, as well as on studies of consumer acceptance and dietary integration necessary to convert regulatory approval into actual nutritional benefits (De Steur et al., 2022).

4.2 Wheat: CIMMYT and Harvest Plus zinc/iron biofortification

CIMMYT's wheat biofortification initiative, which has been under the guidance of HarvestPlus for more than ten years, has methodically examined wild wheat relatives, synthetic hexaploid wheats, and landraces like the zinc-rich 'Zinc-Shakti' accession for increased levels of zinc and iron in grains. This genetic diversity has been utilized to establish both QTL mapping populations and genomic-selection training populations (Rathan et al., 2021; Velu et al., 2020). Through QTL mapping of a Kachu × Zinc-Shakti recombinant inbred line population, assessed over three growing seasons at CIMMYT's Ciudad Obregón station, seven novel pleiotropic QTL for grain zinc and iron were discovered on chromosomes 1B, 1D, 2B, 6A, and 7D. Several of these have been transformed into KASP markers usable by breeders (Rathan et al., 2021). Additionally, high-density GWAS panels, genotyped with 660K and 90K SNP arrays, have identified twenty-five stable zinc loci and sixteen stable iron loci. A locus on chromosome 3A was validated in a separate biparental population and is associated with a MATE-family transporter that is orthologous to the rice gene OsPEZ2 (Tong et al., 2022). This research has already contributed to the development of CIMMYT-derived wheat lines with elevated grain zinc concentrations that have been released as varieties in wheat producing regions through the Harvest Plus programme.

4.3 Pearl millet: ICRISAT's Dhanashakti programme

Since 2004, ICRISAT has led the biofortification of pearl millet with support from Harvest Plus, showcasing an effective blend of traditional and genomic methods. This success is largely due to the trait's high heritability and mainly additive genetic influence. By utilizing the genetic diversity within the commonly cultivated open-pollinated variety ICTP 8203, breeders developed a higher-iron variant named 'Dhanashakti.' This variant not only increased iron content by about 9% compared to its parent but also offered an 11% boost in grain yield, leading to its adoption by tens of thousands of farmers in India (Govindaraj et al., 2016). Concurrently, high-iron hybrid parents were developed from primary breeding lines, resulting in hybrids like ICMH 1201 and ICMH 1301. These hybrids matched Dhanashakti's iron levels but offered a 33 to 38% increase in grain yield, overcoming the yield limitations that have hindered biofortification in other crops (Govindaraj et al., 2019). By the early 2020s, around twelve biofortified pearl millet varieties had been introduced in India and West Africa. Additionally, diagnostic SNP panels for high-iron haplotypes, developed alongside drought-tolerance markers, have been integrated into early-generation screening to expedite the breeding process (Govindaraj et al., 2024; Govindaraj and Pujar, 2023).

4.4 Maize: Quality Protein Maize and Provitamin A stacking

Maize biofortification breeding has advanced to the stage where the routine use of marker-assisted techniques allows for the stacking of provitamin A alleles (crtRB1, lcyE) and protein-quality alleles (o2, o16) within elite hybrid backgrounds that are adapted to specific regions, including specialty types like sweet corn (Zunjare et al., 2018; Hossian et al., 2025). This strategy of stacking multiple traits, achieved through either sequential or simultaneous marker-assisted backcrossing with foreground selection at each target locus and background selection to recover the recurrent-parent genome, illustrates the broader trend of integrating several nutritional traits into single high-yielding products, rather than releasing varieties focused on a single nutrient (Straeten et al., 2020).

4.5 Cassava: The Bio-Cassava Plus programme

Cassava biofortification has focused on the BioCassava Plus initiative, which aimed to enhance provitamin A content in storage roots through transgenic methods, due to the crop's limited genetic diversity, lengthy breeding cycle, and insufficient natural carotenoid variation for traditional selection methods (Sayre et al., 2011). Research on transgenic lines with enhanced provitamin A has shown that while increased carotenoid levels can

extend the shelf life of storage roots, they also affect the distribution of carbon between starch and carotenoid production, leading to a decrease in dry-matter content in certain lines. This trade-off has been addressed in subsequent breeding cycles by selecting among independent transformation events and, more recently, by integrating conventional breeding with genome-editing techniques (Beyene et al., 2018).

4.6 Common bean: Iron and Zinc Biofortification

In the biofortification of common beans, studies have integrated diallel and QTL mapping of Mesoamerican and Andean germplasm pools, which show systematic differences in iron and zinc density (with Andean lines generally having higher iron and Mesoamerican lines higher zinc), alongside GWAS in diverse association panels. Meta-QTL analysis has consistently identified chromosome Pv06 as a reliable zinc hotspot and associated PvZIP17 and PvZIP19 with seed iron/zinc accumulation. Comparative QTL and GWAS studies in biparental Mesoamerican populations specifically bred for high iron/zinc have pinpointed numerous loci for seed mineral concentration. Some of these loci overlap with, and occasionally counteract, yield-component QTL, highlighting the ongoing iron/zinc-yield trade-off observed across bean breeding populations (Diaz et al., 2022; Huertas et al., 2022).

5. Genome Engineering and Transgenic Strategies for Nutritional Enhancement

Conventional breeding is constrained by narrow natural genetic variation and require extended generation times, while precise genome editing technologies provide essential tools for rapid crop nutritional improvement.

Golden Rice and the BioCassava Plus provitamin A cassava stand out as the most significant instances of early transgenic biofortification efforts. These initiatives involve the incorporation of one or more foreign genes to restore or boost a metabolic pathway that is either missing or not sufficiently active in the plant's genome (De steur et al., 2022; Beyene et al., 2018). The advent of CRISPR-Cas genome editing has greatly broadened the range of tools available, allowing for precise alterations to a plant's own genes instead of introducing external DNA. Documented applications relevant to biofortification span diverse crops like rice, wheat, barley, maize, potato, and tomato, include modifications to metal-transporter genes such as OsNAS2 and members of the ZIP family to improve iron and zinc bioavailability. Alterations to storage-protein loci to increase essential amino acid levels, adjustments to carotenoid pathway regulators to boost provitamin A in tomatoes and other horticultural crops, and changes to antinutrient biosynthesis genes (such as phytic acid and tannins) to enhance mineral bioavailability without diminishing the nominal mineral content (Kumar et al., 2022; Jena et al., 2025; Angon et al., 2026).

Base editing, a technique derived from CRISPR, allows for precise single-nucleotide changes without causing double-strand DNA breaks or inserting foreign DNA. This method is particularly significant for biofortification as it can replicate or adjust the effects of naturally beneficial alleles. This is similar to introducing a favorable SNP, like those associated with crtRB1 or NAM-B1 variations, into elite backgrounds that do not possess them. In several regions, including the United States and Japan, this approach faces less regulatory scrutiny compared to traditional transgenesis because the final product does not contain foreign DNA (Jena et al., 2025; Tuncel et al., 2023). This regulatory difference has significantly sped up the field-testing process for several gene-edited nutrient-enhancement prototypes compared to the lengthy two-decade approval process that Golden Rice underwent. However, public and regulatory acceptance still varies widely across countries and remains a dynamic area of policy development rather than a resolved issue (Tuncel et al., 2023). (Table. 2).

6. Multi-Omics, Pan-Genomics, and AI-Assisted Breeding

Recent evaluations in the field highlight a transition from the discovery of individual genes or QTLs to a more comprehensive approach that integrates multiple omics techniques. This involves combining genomics with transcriptomics, metabolomics, and, when possible, ionomics (which involves profiling the elemental composition of entire plants) to develop more thorough mechanistic models of nutrient absorption, transport, and grain accumulation (Lozano and Diaz, 2022; Azeem et al., 2025). For instance, by linking metabolomic profiling of diverse germplasm collections with genomic association studies, researchers have been able to identify which among several candidate genes located within a QTL interval is more likely to be the causal factor, thereby refining the target area for marker development (Lozano and Diaz, 2022).

Pan-genome resources, which encompass structural variations and presence/absence polymorphisms across numerous sequenced accessions of a species rather than depending on a single reference genome, are increasingly recognized as a method to uncover nutrient-trait-associated variations that might be overlooked by reference-genome-based GWAS. This is particularly true for variations found in wild relatives, landraces, and crop wild relatives that are not well represented in a single reference genome assembly (Tiozon et al., 2026; Xu et al., 2024). Simultaneously, artificial intelligence and machine learning techniques are being integrated as genomic prediction models within genomic selection frameworks and as instruments for analyzing multi-omics datasets to identify priority candidate genes and haplotypes for breeding (Azeem et al., 2025; Sandhu et al., 2022). Although not yet a fully established element in operational breeding programs, especially outside major CGIAR and national research institutions, this merging of pan-genomic resources with AI-driven prediction is frequently highlighted in recent review literature as the most promising path for advancing the next generation of multi-nutrient, climate-resilient biofortified varieties.

7. Bioavailability, Nutrient Retention, and Consumer Acceptance

Increases in nominal grain nutrient concentration do not inherently lead to enhanced human nutritional status, a discrepancy that has influenced both breeding priorities and the broader biofortification research agenda. Bioavailability, which refers to the portion of a nutrient that is actually absorbed and used by the human body, is significantly affected by the presence of antinutrients, primarily phytic acid, which binds iron and zinc in the gut and greatly diminishes their absorption. Breeding programmes have therefore combined enhanced mineral-density traits with low phytate alleles or complementary post-harvest processing strategies to improve the actual nutritional benefits, rather than using grain mineral concentration alone as the primary selection criteria (Liu et al., 2026).

Studies on human feeding and efficacy have been crucial in confirming the validity of certain biofortified products. For instance, research on the conversion of β-carotene from Golden Rice into retinol showed an effective transformation that could significantly meet vitamin A needs. These findings were fundamental in building the regulatory case for its approval (Dubock, 2021; De Steur et al., 2022). Similarly, studies confirming the bioavailability of iron in biofortified pearl millet have emphasized the health benefits achieved through breeding improvements (Govindaraj and Pujar, 2023).

Acceptance by consumers and farmers represents another non-genetic factor influencing impact. The history of Golden Rice exemplifies this: although the breeding and safety science issues were resolved over a decade ago, debates surrounding genetic modification postponed its cultivation approval until 2021. In contrast, biofortified crops like OFSP and Dhanashakti pearl millet, which are not genetically distinct from regular varietal releases in regulatory terms, encountered fewer adoption challenges aside from the usual agronomic and market-access issues faced by any new variety (De Steur et al., 2022; Dubock, 2021). Post-approval research on Golden Rice has consequently pivoted toward consumer-acceptance studies and strategies for integrating the trait into preferred localvarietal backgrounds and food systems, an explicit recognition that regulatory approval is a necessary but not sufficient condition for nutritional impact (De Steur et al., 2022).

8. Limitations and Trade-offs in Nutritional Improvement

8.1 Yield and Agronomic Trade-offs

Across various crops and breeding populations, including common bean, negative phenotypic correlations between nutrient density and grain yield or other agronomic traits are frequently observed. In common bean, QTL hotspots for Fe/Zn accumulation often coincide with yield components, showing opposing allelic effects (Diaz et al., 2022). Similarly, in early quality protein maize, the initial opaque2 mutation resulted in a significant penalty on yield and grain quality, which was later partially alleviated through the introduction of modifier genes (Rojas et al., 2010). The primary breeding goal has shifted from merely mapping the nutrient trait to overcoming this trade-off. This is being tackled by selecting favorable recombinants that separate co-located loci, identifying high-yielding parents that incidentally possess beneficial nutrient alleles (as seen in the pearl millet "fast-track" strategy), and employing multi-trait genomic selection indices to simultaneously enhance yield and nutrient density (Krishnappa et al., 2022; Gorafi et al., 2018).

8.2 Genotype -by- Environment Interaction

Grain mineral accumulation is influenced by environmental conditions, including soil micronutrients, moisture regime, and other site-specific factors. Consequently, QTLs or haplotypes that appear robust in a specific location or season may exhibit reduced or inconsistent effects across environments. Multi-environment and multi-season evaluation is critical for confirming the stability and breeding value of nutrient-associated loci before their deployment in marker-assisted selection, although such extensive testing is considerably more resource intensive than conventional single-location yield evaluation (Wang et al., 2021; Gupta et al., 2022).

8.3 Multi-nutrient Stacking Complexity

As breeding priorities shift from single-nutrient to multi-nutrient biofortified products, the complexity of marker-assisted stacking and the risk of unfavourable linkage or epistatic interaction among target loci increase substantially, a challenge repeatedly flagged as unresolved for most staple crops beyond maize's relatively mature crtRB1/lcyE/o2/o16 stacking pipeline (Tiozon et al., 2026).

9. Future Perspectives and Conclusion

Over the past ten years, the path of development indicates that the upcoming stage of biofortification, driven by genetics and genomics, will focus less on identifying new single-gene targets. This task has already progressed significantly for key minerals, vitamins, and amino acids in main staple crops. Instead, the emphasis will shift towards incorporating existing genomic tools into breeding processes that are faster, more accurate, and more comprehensive. Based on recent literature trends, three main directions are anticipated to influence the period from 2026 to 2030.

Firstly, the ongoing merging of genomic selection with rapid breeding techniques and high-throughput, cost-effective phenotyping methods (such as portable XRF, calibrated NIR, and imaging-based ionomics) is predicted to reduce the time from discovering traits to releasing new varieties. This will particularly aid crops like minor millets and legumes, which currently lack the genomic infrastructure available for major cereals (Ceran et al., 2024; Ratna and Caesar, 2026). Secondly, the use of pan-genome and multi-omics resources is anticipated to reveal nutrient-trait variations that remain "hidden" and are segregating within crop wild relatives and underutilized landraces. This approach will enhance the allelic diversity available to breeders, going beyond what has been achieved with single-reference-genome GWAS panels to date (Tiozon et al. 2026, ; Farooq et al., 2026). Thirdly, base editing and other precise genome-editing techniques, particularly those capable of

mimicking the effects of known advantageous natural alleles without incorporating foreign DNA, are expected to shorten the lengthy approval process for transgenic products like Golden Rice. This will allow for a faster deployment path similar to that of conventionally bred products, contingent upon the evolving national regulatory frameworks (Jena et al., 2025; Tuncel et al., 2023).

Achieving these technical advancements as tangible enhancements in the nutritional status of populations will, as consistently highlighted in the literature, continue to rely equally on non-genetic elements. These include effective seed systems that provide biofortified varieties to smallholder farmers, agronomic practices that maintain nutrient benefits under actual field conditions, food-system and market incentives that prioritize nutrient density over yield or grain appearance alone, and ongoing public engagement to foster consumer acceptance, particularly for transgenic strategies and gene-edited products (De Steur et al., 2022; Tiozon et al., 2026).

Over the past two decades, focused genetic and genomic research has transitioned crop biofortification from a promising idea to a practical breeding goal, resulting in the release of farmer-cultivated varieties of staples such as rice, wheat, pearl millet, maize, cassava, and common bean. The years from 2015 to 2026, in particular, witnessed the development of genomic selection and genome-wide association techniques, the publication of crucial reference genomes that revolutionized marker discovery, the long-anticipated regulatory approval of Golden Rice, and the initial significant uses of CRISPR-Cas genome editing for nutrient-density traits. The primary issue is not so much a lack of scientific knowledge as it is the challenge of implementing and integrating solutions. This involves combining various nutrient and agronomic characteristics into single, high-yielding, climate-resilient crop varieties; effectively expanding genomic tools to crops and breeding programs that have historically lacked resources; and ensuring that the genetic advancements made in research stations are converted into tangible reductions in global hidden hunger through seed systems, agronomy, and consumer acceptance.

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AUTHOR CONTRIBUTIONS

Srilakshmi Gonna conceptualized the article and drafted the main manuscript with Anandhi K. Manivannan N., V. Babu Rajendra Prasad and L. Rajendran examined the manuscript's writing format and references. Anandhi K. supervised the entire writing process of the manuscript. All the other authors reviewed the manuscript

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This work does not contain any studies involving human and animal subjects

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

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Figure 1: Conceptual overview of the three complementary routes to crop biofortification (agronomic, genetic, and transgenic/genome-editing) and their convergence on a shared breeding, evaluation, and deployment pipeline.

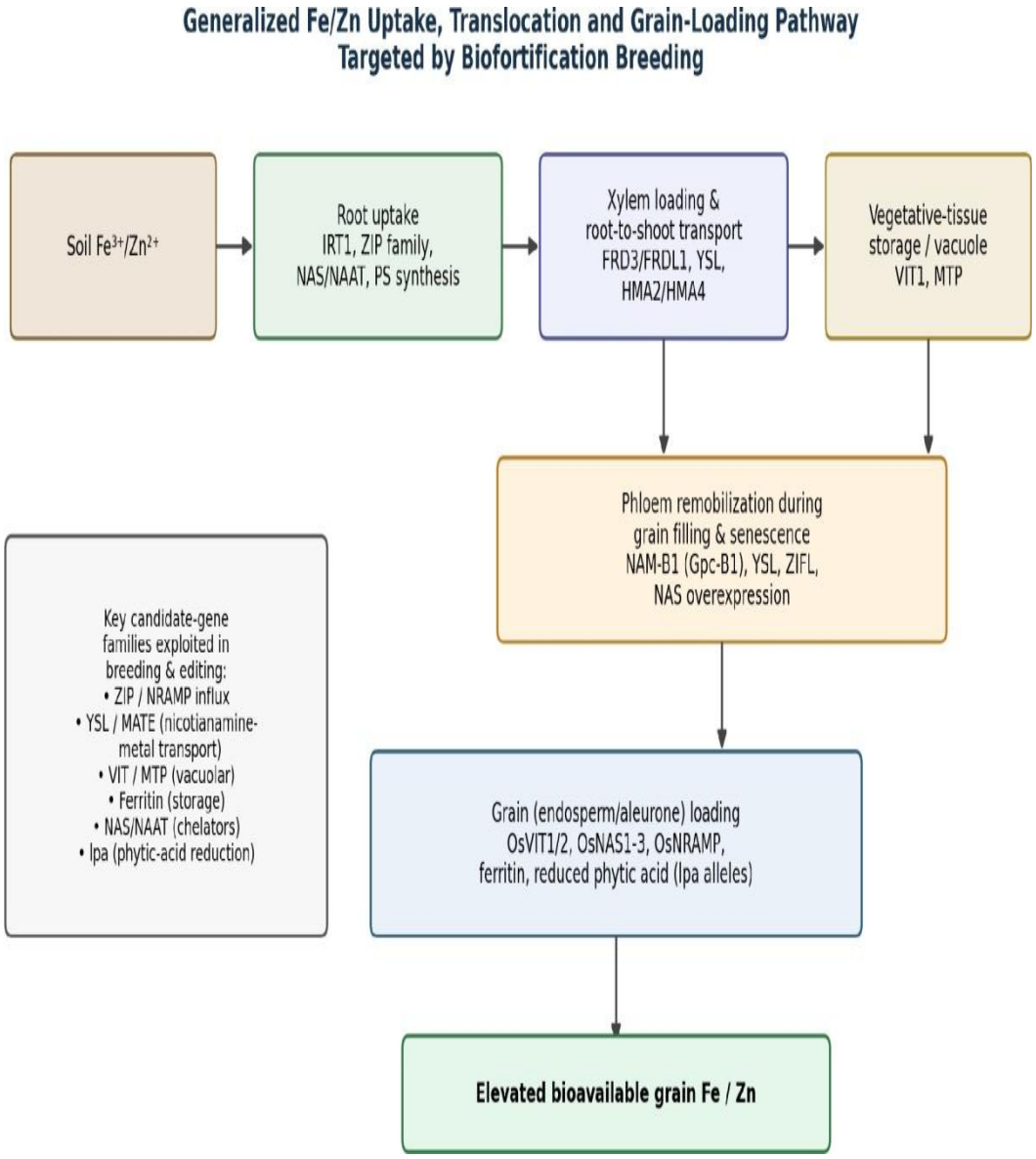

Figure 2: Generalized pathway of iron and zinc uptake, xylem transport, vegetative storage, phloem remobilization, and grain loading, showing candidate-gene families most frequently targeted by biofortification breeding and genome editing across cereals and legumes.

Crop	Target nutrient(s)	Key genes/QTL	Principal breeding approach	Representative outcome
Rice	Provitamin A	psy1(maize), crtI (bacterial) endosperm carotenoid pathway	Transgenesis (GR2 Event)	Golden Rice approved for cultivation in Philippines (2021), Bangladesh. The GR2E construct containing maize *psy* and bacterial *crtI* substantially increased endosperm carotenoids/provitamin A relative to the original Golden Rice (Paine et al. 2005).
Rice	Iron, zinc	OsNAS1–3, OsVIT1/2, OsNRAMP1/7, OsMTP1/6, qZn7.1	QTL mapping, MAGIC population GWAS gene/QTL pyramiding	MAGIC/GWAS studies have identified Fe/Zn-associated loci and candidate genes, including *OsMTP6, OsNAS3, OsVIT1* and *OsNRAMP7* Gene/QTL-pyramided lines with elevated grain Zn and acceptable yield have been developed as breeding resources (Descalsota et al., 2018).
Wheat	Zinc, iron	Gpc-B1/NAM-B1; chromosome2, 7,3A, 7A loci	GWAS (HarvestPlus panel), MABC, KASP marker deployment	CIMMYT biofortified wheat lines released in South Asia (Velu et al., 2018).
Pearl millet	Iron	High-heritability additive loci; diagnostic Fe-haplotype SNPs	Intra-population selection MAS, hybrid-paren screening	Dhanashakti OPV and ICMH 1201/1301 hybrids(India) (Srivastava et al., 2021).
Maize	Provitamin A lysine, tryptophan	crtRB1, lcyE, opaque2 opaque16	Marker-assisted gene stacking/backcrossing	Reconstituted QPM and sweet-corn hybrids
Cassava	Provitamin A	Deregulated phytoene synthase (transgene)	Transgenesis (BioCassavaPlus)	Provitamin A-enhanced storage-root lines, Sub-Saharan Africa
Common bean	Iron, zinc	PvZIP17, PvZIP19, Pv06 hotspot loci	QTL mapping, GWAS MAS	CIAT/HarvestPlus biofortified bean varieties
Sweet potato	Provitamin A	Simple, largely conventional-breeding-tracta ble genetics	Conventional selection/breeding	Orange-fleshed sweet potato, ~7 million households

Table 1: Representative genetics and genomics milestones in biofortification across major staple crops (2015–2026)

Strategy	Underlying principle	Key advantages	Key limitations
Marker-assisted selection backcrossing (MAS/MABC)	Track major-effect alleles with linkedor diagnostic Markers during introgression	Precise; well-suited to single/few major genes; widely validated(crtRB1,o2,NAM-B1) (Collard and Mackill, 2008).	Inefficient for many small-effect loci; linkage drag possible (Collard and Mackill, 2008).

Genome-wide Association studies (GWAS)	Exploit historical Recombination across diverse panels to map trait-marker associations	High mapping resolution leverages existing diversity panels (Juliana et al., 2022; Descalsota et al., 2018).	Requires large, well-phenotyped panels; population-structure confounding (Tong et al., 2022; Pujar et al. 2020).
Genomic selection (GS)	Genome-wide markers used to predict breeding values without per-locus tracking	Effective for low-heritability, polygenic, costly-to-phenotype traits; accelerates cycle time (Habyarimana et al., 2025; Jighly et al., 2019).	High genotyping cost at scale; risk of narrowing diversity for untargeted traits (Habyarimana et al., 2025; Jighly et al. 2019).
Speed breeding + GS integration	Compress generation time via controlled photoperiod/temperature, combined with GS-based parent selection	Maximizes genetic gain per unit time (Samantara et al., 2022; Gudi et al., 2022; Joghly et al., 2019).	Requires controlled-environment infrastructure; species-specific protocols (Samantara et al., 2022; Gudi et al. 2022).
Transgenesis	Introduce heterologous gene(s) to reconstitute or enhance a pathway absent/limiting in the crop	Enables traits with no natural Intra specific variation(e.g., rice cassava provitamin A) (Talsma et al., 2017; Ganguli et al., 2022).	Lengthy, costly, country-specific regulatory approval variable public acceptance) (Talsma et al., 2017 Ganguli et al., 2022).
CRISPR-Cas genome editing (incl. base editing)	Precisely modify the crop's own gene sequence, often without retaining foreign DNA	Faster regulatory pathway in several jurisdictions; can recreate known beneficial Natural alleles (Mishra et al. 2019; Nerkar et al., 2022).	Off-target effects possible; Regulatory status still evolving in many countries (Mishra et al., 2019; Sturme et al., 2022).

Table 2: Comparison of principal breeding and biotechnological strategies used in crop biofortification