



Review Article

Implementing Marker-Assisted Selection for Biofortification of Staple Crops With Iron, Zinc, and Provitamin A: A Review

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Abstract

Micronutrient malnutrition remains a major global nutrition problem, particularly in populations whose diets depend heavily on staple crops. Iron (Fe), zinc (Zn), and vitamin A deficiencies can adversely affect growth, immunity, cognitive development, and overall health. Crop biofortification provides a sustainable breeding-based strategy for increasing the concentration of essential nutrients in commonly consumed foods. This review examines the application of marker-assisted selection (MAS) in the development of Fe-, Zn-, and provitamin A (PVA)-rich staple crops. Relevant peer-reviewed literature, scientific reviews, and institutional publications were examined using major academic databases and search platforms, including PubMed, Scopus, Web of Science, and Google Scholar. Particular attention was given to the genetic basis of micronutrient accumulation, molecular markers, marker-assisted backcrossing, marker-assisted recurrent selection, gene pyramiding, and recent applications in maize, rice, wheat, pearl millet, cassava, and other staple crops. Evidence from breeding programs indicates that MAS can improve the efficiency of selecting favorable alleles and can facilitate the introgression and combination of nutrient-associated genes and quantitative trait loci (QTLs). Major targets include *crtRB1* and *lcyE* for PVA accumulation in maize, genomic regions associated with grain Fe and Zn in cereals, and nutrient-related loci in rice and cassava. Nevertheless, the effectiveness of MAS can be constrained by the quantitative inheritance of micronutrient traits, genotype × environment interactions, marker–gene recombination, limited genomic resources in some crops, and differences in nutrient bioavailability. Future breeding strategies should integrate MAS with genomic selection, high-throughput phenotyping, speed breeding, gene editing, and climate-resilient breeding. Overall, MAS represents an important component of modern biofortification programs and can contribute to the development of nutritionally improved, productive, and farmer-acceptable staple crop varieties.

Keywords

Bioavailability, Biofortification, Marker-assisted Selection, Micronutrients, Staple Crops

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Received: 11 August 2026; Accepted: 22 August 2026; Published: 9 September 2026



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1. Introduction

Micronutrient malnutrition, commonly described as “hidden hunger,” remains a major challenge to human health and food security. Populations that rely heavily on cereal- and root-based diets may consume sufficient calories while receiving inadequate amounts of essential micronutrients. Among the most important deficiencies are iron (Fe), zinc (Zn), and vitamin A deficiency, which are associated with anemia, impaired immune function, poor growth and development, and other adverse health outcomes [4, 9, 10].

Conventional crop breeding has contributed substantially to increasing the nutritional quality of staple crops. However, nutritional traits can be difficult to select because their expression is affected by genotype, environment, developmental stage, and interactions among multiple genes. Molecular breeding approaches provide additional tools for identifying favorable alleles and accelerating their incorporation into elite germplasm [5, 18].

Biofortification is particularly attractive because it increases the nutritional value of crops during production rather than relying exclusively on postharvest processing or supplementation. International breeding programs have developed and disseminated Fe-, Zn-, and PVA-enhanced varieties of maize, wheat, rice, beans, cassava, sweet potato, millet, and other crops [9, 10, 21]. The transition from targeted biofortification breeding toward mainstreaming micronutrient traits into conventional breeding pipelines has further increased the potential scale of this approach [21]. Evidence from global breeding programs demonstrates that biofortification can be integrated with yield, disease resistance, and adaptation objectives rather than being treated as an independent breeding target [21].

Marker-assisted selection (MAS) uses DNA markers associated with genes or genomic regions controlling desirable traits to identify superior breeding materials. In contrast to selection based exclusively on phenotype, MAS allows breeders to make selection decisions using information about the genotype of individual plants. This is particularly useful for traits that are difficult, expensive, or environmentally sensitive to measure [5, 6].

Several molecular breeding strategies have been used to improve micronutrient concentration. These include marker-assisted backcrossing (MABC), marker-assisted recurrent selection (MARS), foreground and background selection, and marker-assisted gene pyramiding. Their application has been particularly promising for PVA accumulation in maize and for Fe and Zn improvement in cereals and other staple crops [7, 16, 20].

Recent developments have expanded the role of molecular breeding beyond conventional MAS. Genomic selection, high-throughput genotyping, speed breeding, and genome editing offer opportunities to improve complex nutritional traits more rapidly [8, 18, 21]. Therefore, understanding how MAS can be integrated with these technologies is important for developing biofortified varieties that combine nutritional quality

with agronomic performance and adaptation.

1.1. Rationale for Marker-Assisted Biofortification

The effectiveness of biofortification depends not only on increasing the concentration of a nutrient but also on delivering that nutrient through crops that are widely consumed and agronomically competitive. Consequently, breeding programs need to combine nutritional targets with yield, adaptation, disease resistance, abiotic-stress tolerance, and consumer preferences [9, 21].

MAS can support this process by enabling breeders to retain favorable nutritional alleles while simultaneously selecting for agronomic characteristics. For example, molecular markers linked to PVA-associated loci can be used during maize improvement, while genomic regions associated with Fe and Zn accumulation can be incorporated into elite cereal backgrounds [7, 20]. Such approaches can reduce the number of generations required to identify desirable breeding materials and can facilitate the combination of several favorable loci.

1.2. Objectives of the Review

1.2.1. General Objective

To review the role and application of marker-assisted selection in the biofortification of staple crops with iron, zinc, and provitamin A.

1.2.2. Specific Objectives

To review the genetic mechanisms underlying Fe, Zn, and PVA accumulation in staple crops.

To examine molecular markers and MAS strategies used for nutritional improvement.

To summarize recent progress in MAS-assisted biofortification of major staple crops.

To identify major technical and biological limitations affecting MAS-based biofortification.

To discuss future opportunities for integrating MAS with genomic and biotechnological approaches.

2. Methodology for Literature Review

This review was developed from scientific literature addressing crop biofortification, molecular breeding, micronutrient genetics, and marker-assisted selection. Peer-reviewed journal articles, review papers, and authoritative scientific publications were considered. Literature searches were conducted using PubMed, Scopus, Web of Science, Google Scholar, and ResearchGate.

Search terms included combinations of the following keywords: “biofortification,” “marker-assisted selection,”

“marker-assisted backcrossing,” “marker-assisted recurrent selection,” “iron biofortification,” “zinc biofortification,” “provitamin A,” “molecular breeding,” “QTL,” “SNP markers,” “SSR markers,” “maize,” “rice,” “wheat,” “cassava,” “pearl millet,” and “staple crops.”

Studies were prioritized when they provided evidence on the genetic basis of nutrient accumulation, identification or validation of molecular markers, application of MAS in breeding populations, development of nutrient-dense varieties, or evaluation of the nutritional relevance of biofortified crops. Publications with insufficient methodological information or limited relevance to crop biofortification were given lower priority.

Information extracted from the selected literature included crop species, target nutrient, genetic factors or QTLs, marker type, breeding strategy, major findings, and reported limitations. The literature was then synthesized thematically according to biofortification approaches, molecular breeding strategies, genetic mechanisms, crop-specific progress, limitations, and future opportunities.

3. Body

3.1. Concept and Importance of Biofortification

Biofortification refers to the development of crop varieties with increased concentrations or improved nutritional quality of essential nutrients through plant breeding, agronomic management, or biotechnology. Unlike conventional food fortification, which generally adds nutrients after harvest or during food processing, biofortification aims to increase the nutritional value of the crop before harvest [10, 11].

The approach is particularly relevant to rural and low-income populations that depend on staple crops for a large proportion of their daily energy intake. Increasing the concentration of Fe, Zn, and PVA in widely consumed crops can therefore improve dietary nutrient intake without requiring major changes in food consumption patterns [9, 21].

Biofortification programs have targeted several crops and nutrients. Fe and Zn enrichment has received considerable attention in wheat, rice, pearl millet, beans, lentils, and other crops, whereas PVA enhancement has been especially important in maize, cassava, sweet potato, and banana [2, 12, 16, 20].

Biofortification may involve conventional selection, agronomic interventions, molecular breeding, transgenic approaches, or combinations of these methods [11, 19]. From a breeding perspective, the desired crop should provide a meaningful contribution to the population's diet so that replacing conventional cultivars with nutrient-dense cultivars produces a measurable nutritional benefit [3].

3.2. Breeding Approaches for Biofortification

Conventional breeding has been an important foundation for crop biofortification. It exploits naturally occurring genetic variation for nutrient accumulation and combines desirable alleles through hybridization and selection. However, phenotypic selection for micronutrient concentration may be relatively slow because nutrient traits are frequently quantitative and influenced by environmental conditions [4, 20].

Molecular breeding approaches can complement conventional breeding by providing information about the genetic factors responsible for nutrient accumulation. MAS is particularly useful when reliable markers are available for genes or QTLs with sufficiently large effects. Genomic selection may provide additional advantages for highly polygenic traits by using genome-wide marker information to predict breeding values [18, 21].

3.3. Marker-Assisted Selection for Biofortification

Marker-assisted selection is a molecular breeding approach in which DNA polymorphisms associated with desirable genes or genomic regions are used to select individuals with favorable genetic profiles. The approach can reduce dependence on phenotypic screening and can be applied at early developmental stages.

The usefulness of MAS depends on the strength and reliability of the association between the marker and the target trait. Markers that are closely linked to causal genes generally provide more reliable selection than markers located at greater genetic distances from the target locus [5, 6].

For biofortification, MAS can be used to select alleles associated with mineral uptake, translocation, storage, carotenoid biosynthesis, and other biological processes that influence nutritional quality. Major strategies include MABC, MARS, foreground selection, background selection, recombinant selection, and gene pyramiding [5].

(i). Marker-Assisted Backcrossing

Marker-assisted backcrossing is primarily used to introduce a target allele or QTL from a donor parent into an elite recipient variety. The approach combines conventional backcrossing with molecular selection.

Three forms of selection are particularly useful. Foreground selection identifies plants carrying the target allele. Recombinant selection can reduce linkage drag surrounding the target locus, while background selection increases the recovery of the recurrent parent genome [5].

MABC has been applied to nutritional improvement in maize and other crops. In maize, molecular markers associated with carotenoid biosynthesis genes such as *crtRB1* have been used to facilitate the development of PVA-enriched germplasm. Recent work has also demonstrated the potential for combining PVA enrichment with heat and drought resilience [5].

(ii). Marker-Assisted Recurrent Selection

Marker-assisted recurrent selection combines recurrent selection with molecular marker information. Instead of transferring one major allele into an elite background, MARS can increase the frequency of favorable alleles at multiple loci over successive selection cycles.

This strategy is particularly appropriate for complex traits controlled by multiple loci. The use of marker-based recurrent selection to increase PVA concentration in tropical maize synthetics [7]. The study illustrates the potential of molecular selection to increase favorable allele frequencies for nutritional traits while continuing population improvement.

(iii). Gene and QTL Pyramiding

Gene pyramiding involves combining multiple favorable genes or QTLs within a single breeding genotype. It is particularly relevant to biofortification because nutrient accumulation can depend on several biological processes, including uptake, transport, metabolism, storage, and remobilization.

MAS facilitates pyramiding because individual plants can be screened simultaneously for several target loci. In PVA maize, genes such as *crtRB1* and *lcyE* have been important targets because they influence carotenoid accumulation in the grain [7, 21]. In rice, multiple genomic regions associated with grain Fe and Zn can similarly be considered during molecular breeding [20].

Pyramiding can therefore provide a means of combining nutritional improvement with resistance, adaptation, and yield-related traits. However, the resulting genotype should be evaluated phenotypically across environments because the presence of favorable alleles does not necessarily guarantee consistent nutrient expression.

3.4. Molecular Markers Used in Biofortification

Several classes of DNA markers have been used in plant breeding, including simple sequence repeats (SSRs), amplified fragment length polymorphisms (AFLPs), and single nucleotide polymorphisms (SNPs) [6].

(i). Simple Sequence Repeat Markers

Simple sequence repeats, also known as microsatellites, are highly polymorphic molecular markers that have been widely used for genetic diversity analysis and marker-trait association studies. SSR markers have been used to investigate genetic variation associated with Fe and Zn concentration in rice and other crops [17].

(ii). Single Nucleotide Polymorphism Markers

Single nucleotide polymorphisms are single-base variations distributed throughout plant genomes. Their high abundance, suitability for high-throughput genotyping, and compatibility with automated platforms make SNPs particularly valuable for modern breeding.

Genome-wide association studies and SNP-based analyses have identified genomic regions associated with Fe, Zn, protein, and other nutritional traits in crops such as pearl millet [16]. SNP markers have also been validated for quality control, diversity assessment, and trait selection in biofortified cassava populations [13].

(iii). Marker Selection and Validation

The practical value of a marker depends on its reproducibility, reliability, genetic distance from the causal locus, and performance across breeding populations. Therefore, markers identified in one population should ideally be validated in independent germplasm before routine deployment.

3.5. Genetic Basis of Iron, Zinc, and Provitamin A Accumulation

The accumulation of Fe, Zn, and PVA is influenced by multiple genetic and physiological processes. These include uptake from the soil, movement within the plant, remobilization, cellular storage, and biochemical synthesis.

1) Iron and Zinc

Grain Fe and Zn concentrations are generally quantitative traits influenced by multiple genomic regions and environmental conditions. In wheat, genes associated with nutrient remobilization and grain accumulation, including *Gpc-B1*-related genomic regions, have received substantial attention [4, 8].

Rice improvement has identified several genomic regions and genes involved in Zn and Fe accumulation. Molecular breeding studies have therefore focused on identifying QTLs and candidate genes that can be incorporated into elite backgrounds [17, 20].

In pearl millet, genome-wide association analyses have identified SNP-associated genomic regions for grain Fe and Zn concentration, providing potential targets for breeding and genomic improvement [16].

2) Provitamin A

Provitamin A carotenoids, particularly β -carotene, are important nutritional targets in several staple crops. In maize, carotenoid concentration is influenced by genes involved in carotenoid biosynthesis and conversion. The *crtRB1* and *lcyE* loci are among the most important targets used in molecular breeding for PVA enhancement [7, 21].

Marker-based selection has been particularly valuable because carotenoid concentration can be measured using laboratory-based analytical procedures that may not be practical for very large breeding populations. Molecular markers can therefore be used to enrich populations for favorable alleles before detailed phenotyping.

3.6. Progress in MAS-Assisted Biofortification of Major Staple Crops

1) Maize

Maize is an important target for biofortification because it is a major staple in many African and Latin American countries. Breeding programs have targeted Fe, Zn, PVA, protein quality, and other nutritional characteristics.

Molecular breeding has been particularly successful for PVA improvement. Marker-based recurrent selection increased PVA carotenoid concentrations in tropical maize populations, demonstrating the potential of MAS for improving complex nutritional traits [7]. Marker-assisted backcrossing has also been used to enrich maize inbreds with PVA while considering heat and drought adaptation [1]. The use of molecular markers for Zn and Fe improvement has additionally provided opportunities to combine several nutritional targets with desirable agronomic characteristics.

2) Rice

Rice is one of the world's most important staple cereals, particularly in Asia. Polishing removes substantial portions of the nutrient-rich outer grain layers, making improvement of mineral concentration an important breeding objective.

Breeding and genomic studies have identified donors, QTLs, and molecular markers associated with grain Zn and Fe concentration. These resources can support MAS and other genomic approaches for developing nutrient-dense rice varieties [17, 20]. Recent reviews indicate that numerous biofortified rice varieties targeting Fe, Zn, protein, and PVA have been developed or released, demonstrating the increasing integration of nutrition traits into rice improvement programs [20].

3) Wheat

Wheat is a major staple crop and an important target for Zn and Fe biofortification. The genetic basis of grain micronutrient accumulation is complex, requiring the integration of conventional breeding with molecular tools.

SNP-enriched linkage mapping has identified genomic regions associated with nutritional traits in wheat, providing potential targets for MAS and genomic selection [8]. The use of

molecular information can help breeders select nutrient-dense lines while retaining yield and adaptation characteristics.

4) Pearl Millet

Pearl millet is particularly important in semi-arid regions where it contributes substantially to food and nutritional security. Its natural variation for grain Fe and Zn provides an important resource for biofortification.

Genome-wide association studies have identified SNP-associated genomic regions for Fe, Zn, and protein concentration in pearl millet [16]. Such genomic resources can support the development of diagnostic markers and improve the efficiency of nutrient-focused breeding.

5) Cassava

Cassava is a major staple in tropical regions and provides a substantial proportion of dietary energy in many African communities. Its nutritional improvement has focused particularly on PVA because cassava roots naturally have low carotenoid concentrations in many varieties.

SNP marker validation in biofortified cassava populations has demonstrated the potential of molecular markers for diversity analysis, quality control, and trait selection [13]. Molecular approaches can therefore complement conventional breeding for the development and maintenance of nutrient-dense cassava populations.

6) Tef and Other Underutilized Crops

Tef (*Eragrostis tef*) is an important Ethiopian cereal with considerable nutritional and cultural value. Compared with major crops such as maize, rice, and wheat, tef has historically had fewer genomic resources. However, advances in molecular breeding and genome editing are expanding the possibilities for genetic improvement of this crop [14].

The development of genomic resources for underutilized and regionally important staples is particularly important for African biofortification programs because local crops may have major roles in regional food systems.

3.7. Summary of MAS-Based Biofortification Studies

Table 1. Examples of molecular breeding applications relevant to Fe, Zn, and PVA biofortification.

Crop	Target nutrient	Molecular approach / target	Major contribution
Maize	PVA	Marker-assisted recurrent selection; PVA-associated markers	Increased carotenoid concentration through successive selection cycles [7]
Maize	PVA	Marker-assisted backcrossing; <i>crtRB1</i> -associated markers	Facilitated introgression of favorable PVA alleles into elite backgrounds [1]
Maize	Fe, Zn, PVA	SSR/SNP-based selection and molecular breeding	Provided opportunities for simultaneous improvement of nutritional traits
Rice	Fe, Zn	QTL mapping, SSR/SNP markers, genomic approaches	Identification of genomic regions and donor materials for grain mineral improvement [17, 20]
Wheat	Fe, Zn	SNP-enriched linkage mapping and molecular breeding	Identification of genomic regions associated with nutritional traits [8]

Crop	Target nutrient	Molecular approach / target	Major contribution
Pearl millet	Fe, Zn	Genome-wide association analysis using SNP markers	Identification of genomic regions associated with grain mineral concentration [16]
Cassava	PVA	SNP marker validation and molecular breeding	Improved molecular resources for trait selection in biofortified populations [13]

3.8. Major Genes and Genomic Regions Associated With Biofortification

Table 2. Examples of genes and genomic regions associated with Fe, Zn, and PVA improvement.

Crop	Nutrient	Gene/QTL or genomic resource	Breeding relevance
Maize	PVA	<i>crtRB1</i>	Associated with carotenoid conversion and PVA concentration [7]
Maize	PVA	<i>lcyE</i>	Important carotenoid-pathway locus used in molecular breeding [21]
Rice	Zn	<i>OsZIP</i> family and other Zn-related loci	Involved in Zn uptake and transport [20]
Rice	Fe/Zn	Multiple QTLs	Provide targets for molecular breeding [17, 20]
Wheat	Fe/Zn	<i>Gpc-B1</i> -related genomic regions	Associated with nutrient remobilization and grain concentration [4, 8]
Pearl millet	Fe/Zn	Multiple SNP-associated regions	Potential targets for genomic and marker-assisted selection [16]
Cassava	PVA	SNP-associated genomic resources	Facilitate selection and quality control in biofortified populations [13]

4. Limitations and Challenges

Despite its potential, MAS is not a universal solution for all biofortification traits. Several biological, technical, and socio-economic factors can limit its effectiveness.

4.1. Quantitative Genetic Control

Fe and Zn concentrations are generally influenced by numerous loci with relatively small effects. Selecting only a few markers may therefore capture only part of the genetic variation underlying the phenotype [4, 8, 20]. For highly polygenic traits, genomic selection may offer advantages because it incorporates information from a much larger number of genomic markers.

4.2. Genotype × Environment Interaction

Micronutrient concentrations can vary substantially among environments. Soil characteristics, climatic conditions, water availability, fertilizer management, and other factors can influence nutrient uptake and accumulation. Consequently, a marker or QTL identified in one population or environment may not have the same predictive value elsewhere [15, 20]. Multi-environment evaluation is therefore essential before releasing biofortified cultivars.

4.3. Marker–Gene Recombination

Markers are not always the causal polymorphisms underlying the target trait. Recombination between a marker and the functional allele can reduce selection accuracy. The closer the marker is to the causal gene, the more reliable marker-based selection is expected to be. Marker validation in independent populations is therefore important before large-scale implementation.

4.4. Limited Genomic Resources

Major crops such as maize, rice, and wheat possess extensive genomic resources. In contrast, some regionally important crops, including tef, may have fewer validated markers and genomic datasets [14]. Limited laboratory infrastructure and genotyping capacity can further restrict MAS implementation in developing-country breeding programs.

4.5. Loss of Genetic Diversity

Strong selection for specific alleles may reduce genetic diversity within breeding populations. This is particularly important when recurrent selection is conducted over several cycles. For example, marker-based recurrent selection for PVA

in maize improved carotenoid concentration but also highlighted the need to manage genetic diversity during selection [7].

Breeding programs should therefore balance nutritional improvement with genetic diversity, yield, adaptation, and resistance to biotic and abiotic stresses.

4.6. Nutrient Bioavailability

A higher nutrient concentration does not automatically translate into a proportional nutritional benefit for humans. Fe and Zn absorption can be influenced by anti-nutritional compounds, particularly phytate and certain polyphenols [4, 15]. Consequently, future biofortification programs should consider both nutrient concentration and bioavailability.

4.7. Cost and Technical Capacity

Although MAS can reduce phenotyping requirements, molecular breeding requires genotyping infrastructure, laboratory facilities, trained personnel, reliable DNA extraction and marker systems, and financial resources. These requirements can be challenging for breeding institutions with limited resources.

5. Future Directions

5.1. Integration With Genomic Selection

Genomic selection (GS) uses genome-wide marker information to predict the breeding values of individuals. Unlike conventional MAS, which generally emphasizes a limited number of markers associated with specific loci, GS can capture the combined effects of many loci. This makes it particularly attractive for quantitative traits such as Fe and Zn concentration [18, 21].

Integrating MAS and GS could allow breeders to use major-effect markers for known genes while simultaneously exploiting genome-wide variation for polygenic components of nutritional traits.

5.2. Integration With Gene Editing

Gene-editing technologies, particularly CRISPR-based systems, provide opportunities to modify genes involved in nutrient uptake, transport, metabolism, storage, and bioavailability. Combining genome editing with MAS could provide complementary strategies for developing nutrient-dense cultivars.

However, regulatory requirements, biosafety considerations, technical limitations, and public acceptance need to be addressed before widespread deployment.

5.3. Speed Breeding and High-Throughput Phenotyping

Speed breeding can shorten generation intervals by optimizing environmental conditions for rapid plant development. When combined with MAS, it can allow breeders to complete several generations within a relatively short period.

High-throughput phenotyping can complement molecular selection by allowing large populations to be evaluated for nutritional and agronomic traits. The combination of genotyping, phenotyping, and accelerated generation advancement is expected to increase genetic gain [21].

5.4. Multi-Trait Breeding

Future biofortified varieties should not be selected for nutrient concentration alone. Farmers require varieties with competitive yield, disease and pest resistance, drought and heat tolerance, appropriate maturity, and acceptable grain or root quality. Therefore, biofortification should increasingly become part of mainstream breeding programs in which nutritional traits are selected simultaneously with agronomic traits [21].

5.5. Improving Bioavailability

Future research should move beyond nutrient concentration toward nutritional effectiveness. Breeding programs should investigate genetic variation in anti-nutritional compounds such as phytate and examine how nutrient density interacts with food processing, cooking, and dietary composition.

Combining high Fe or Zn concentration with improved bioavailability could increase the actual nutritional benefit of biofortified crops [4, 15].

5.6. Climate-Resilient Biofortification

Climate change may influence both crop productivity and nutrient accumulation. Therefore, future biofortification programs should target varieties that maintain nutritional quality under drought, heat, irregular rainfall, and other environmental stresses. Integrating MAS with climate-resilient breeding can help ensure that nutritional improvement remains effective under changing production environments.

5.7. Systems Biology and Multi-Omics

Genomics, transcriptomics, proteomics, metabolomics, and phenomics can provide complementary information about the biological mechanisms controlling micronutrient accumulation. Integrating these data can improve identification of candidate genes, regulatory pathways, and molecular markers.

Such approaches may ultimately allow breeders to develop more predictive models of nutrient accumulation and improve selection efficiency for complex nutritional traits.

6. Conclusion

Marker-assisted selection has become an important component of modern crop biofortification. Its principal advantage is the ability to use molecular information to identify and retain favorable alleles while reducing some of the limitations associated with phenotype-only selection. Applications in maize, rice, wheat, pearl millet, cassava, and other crops demonstrate its potential for improving Fe, Zn, and PVA concentration.

Nevertheless, MAS should not be considered a stand-alone solution. The quantitative inheritance of many micronutrient traits, genotype $\times$ environment interactions, marker–gene recombination, limited genomic resources, and variation in nutrient bioavailability can reduce its effectiveness. Successful breeding programs should therefore integrate MAS with conventional breeding, genomic selection, high-throughput phenotyping, speed breeding, and, where appropriate, genome-editing technologies.

Most importantly, future biofortification should focus on nutritionally meaningful products that combine high nutrient concentration with improved bioavailability, yield, adaptation, stress resistance, and farmer and consumer acceptance. Mainstreaming nutritional traits into broader crop improvement programs offers a promising pathway for expanding the impact of biofortification and contributing to long-term nutritional security [21].

Abbreviations

AFLP	Amplified Fragment Length Polymorphism
Fe	Iron
GS	Genomic Selection
G $\times$ E	Genotype $\times$ Environment Interaction
MABC	Marker-Assisted Backcrossing
MAS	Marker-Assisted Selection
MARS	Marker-Assisted Recurrent Selection
PVA	Provitamin A
QTL	Quantitative Trait Locus
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeat
Zn	Zinc

Author Contributions

Alemayehu Latera: Conceptualization, Data curation, Investigation, Methodology, Resources, Visualization, Writing – original draft, Writing – review & editing

Ermias Estifanos: Conceptualization, Methodology, Resources, Supervision, Validation, Writing – review & editing

Conflicts of Interest

The authors declare no conflicts of interest.

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