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Integrated Approaches for Zinc Biofortification in Pulse Crops: Connecting Genetic Improvement, Precision Agronomy, and Biotechnological Innovations for Sustainable Nutritional Security

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Abstract— *Zinc (Zn) deficiency remains a critical global nutritional challenge, especially in regions where populations rely heavily on plant-based diets. Pulse crops are promising targets for zinc biofortification because of their nutritional value, wide consumption, and role in sustainable agriculture. However, improving zinc accumulation in pulses is a complex process influenced by genetic variation, soil properties, environmental conditions, and crop management practices. This review highlights an integrated approach that connects genetic improvement, precision agronomy, biotechnology, and microbial-based strategies for sustainable zinc biofortification. Genetic approaches, including conventional breeding, marker-assisted selection, genomic tools, and high-throughput phenotyping, provide opportunities to identify zinc-efficient genotypes. Precision agronomic practices, such as site-specific zinc management, foliar application, and advanced fertilizer systems, improve zinc availability and uptake under diverse soil conditions. In addition, zinc-solubilizing bacteria, plant growth-promoting rhizobacteria, and arbuscular mycorrhizal fungi enhance rhizosphere zinc mobilization and support plant nutrient acquisition. Emerging biotechnological tools, including genome editing, transcriptomics, and metabolomics, further improve understanding of zinc transport, regulation, and storage mechanisms. Despite significant progress, challenges remain, including genotype × environment interactions, limited field validation, variable microbial performance, and barriers to large-scale adoption. Therefore, a single approach cannot provide consistent zinc enrichment across different agricultural systems. An integrated gene-to-field framework combining genetic potential, environmental information, optimized management, and biological enhancement is essential for achieving stable zinc-rich pulse production. Future research should focus on multidisciplinary approaches that translate molecular discoveries into practical agricultural solutions. Such integration can contribute to improved crop nutritional quality, sustainable production systems, and long-term strategies for reducing micronutrient deficiency worldwide.*

Keywords— ***Zinc biofortification; Pulse crops; Micronutrient deficiency; Genetic improvement; Precision agronomy; Plant growth-promoting microorganisms; Zinc-solubilizing bacteria; Genome editing; Rhizosphere engineering; Nutritional security***

I. INTRODUCTION

Zinc (Zn) deficiency is actually among the most prominent micronutrient issues of the human health, especially among populations with a high reliance on cereal and plant-based diets. As zinc is involved in the immune system, growth and metabolism of cells it causes increased susceptibility to infection and reduced development. Programs of supplementation and fortification of food have been

effective, but are restricted by the cost, delivery system and ongoing interventions. Crop based biofortification works around the problem instead by increasing the nutrient levels of already grown and consumed foods. Pulses are particularly suitable target for biofortification programs because they provide important nutritional benefits, including high-quality protein, dietary fiber, and essential minerals. In addition, their ability to fix atmospheric nitrogen contributes to soil fertility and sustainable agricultural practices. Since pulses are widely consumed in regions where nutritional deficiencies and agricultural challenges are prevalent, biofortification of these crops can offer both dietary and agronomic advantages (Lakshmanan et al., 2025; Hasanuzzaman et al., 2023) (J et al., 2025). The germplasm of pulses is genetically diverse for Zn accumulation and breeding program have been used to select high zinc lines. However, zinc accumulation is not controlled by a few genes, it is influenced by soil chemical properties and the management of the crop in terms of root uptake, internal transport, and loading of zinc in the seeds. The performance of a genotype largely depends on soil conditions. Even high-performing genotypes may show limited zinc accumulation when grown in zinc-deficient or high-pH soils. Similarly, applying excessive amounts of zinc fertilizer may not improve zinc content if the selected genotype has a poor capacity for zinc uptake and accumulation. This means that soil and crop specific information will be needed to support precision agronomy as an essential complement to crop improvement via plant breeding. Recent advances in genome editing, transcriptomics, and metabolomics have improved our understanding of the mechanisms involved in zinc uptake, transport, and accumulation from soil to seeds. In addition, beneficial microorganisms such as zinc-solubilizing bacteria, plant growth promoting rhizobacteria, and mycorrhizal fungi offer a sustainable and low-input approach to enhance zinc availability in the rhizosphere (Tessema et al., 2026; Shahzad et al., 2021) (J et al., 2026). However, these research areas are often studied separately rather than through an integrated approach. Breeding programs, fertilizer experiments, and microbiome or molecular studies usually focus on individual aspects of zinc improvement. As a result, they address only specific parts of the problem and may overlook the interactions between genetic, agronomic and biological factors. This review integrates these complementary research area into unified framework. This reviews the zinc flow through the soil-plant-seed pathway, then explores genetic, agronomic, biotechnological and microbial approaches to enhancing zinc nutrition, and finally presents a gene to fields framework that will integrate the genetic potential, agronomic management, biological enhancement and molecular tools. The objective here is not to introduce an additional stand-alone technology but to provide a description of how pulse breeding and production can lead to a meaningful and stable zinc enriched product (Tessema et al., 2026; Lakshmanan et al., 2025) (J et al., 2025).

II. ZINC UPTAKE, TRANSPORT AND ACCUMULATION MECHANISMS IN PULSE CROPS

Accumulation of zinc in the seeds of pulses is a complex process of root uptake from soil, translocation through the plant and deposition in developing grains. The activity of the transporters and the physiology of the seeds and soil chemistry all have individual effects on each step, and enhancing one without the others is unlikely to lead to the desired improvement. A clear understanding of the soil–plant–seed continuum provides the basis for integrating breeding, agronomy, and biotechnology as complementary approaches rather than treating them as separate solutions. This interconnected perspective forms the biological foundation of the integrated framework developed throughout this review. The current molecular knowledge about zinc uptake, transport and loading into seeds has been derived from cereal crops and less well characterized model plant systems such as rice, barley, Arabidopsis, and others. While these studies offer important frameworks for the mechanistic understanding, functional validation of Zn transport pathways in pulse crops is relatively scarce. Thus molecular information gained from cereals and model organisms should be treated as a theory and/or mechanism, not necessarily an equivalent to pulse zinc biology. It is crucial to expand functional studies of pulse crops, such as common bean, chickpea, lentil and pea, to create crop-specific biofortification strategies. A successful zinc biofortification strategy requires an understanding of how zinc moves through the soil–plant–seed continuum. **Figure 1** summarizes the major processes involved in zinc availability, root uptake, transport, and seed accumulation in pulse crops. The figure highlights that zinc enrichment is controlled by multiple interconnected steps, and limitations at any stage can influence the final zinc concentration and nutritional value of the grain (Hasanuzzaman et al., 2023; Lakshmanan et al., 2025).

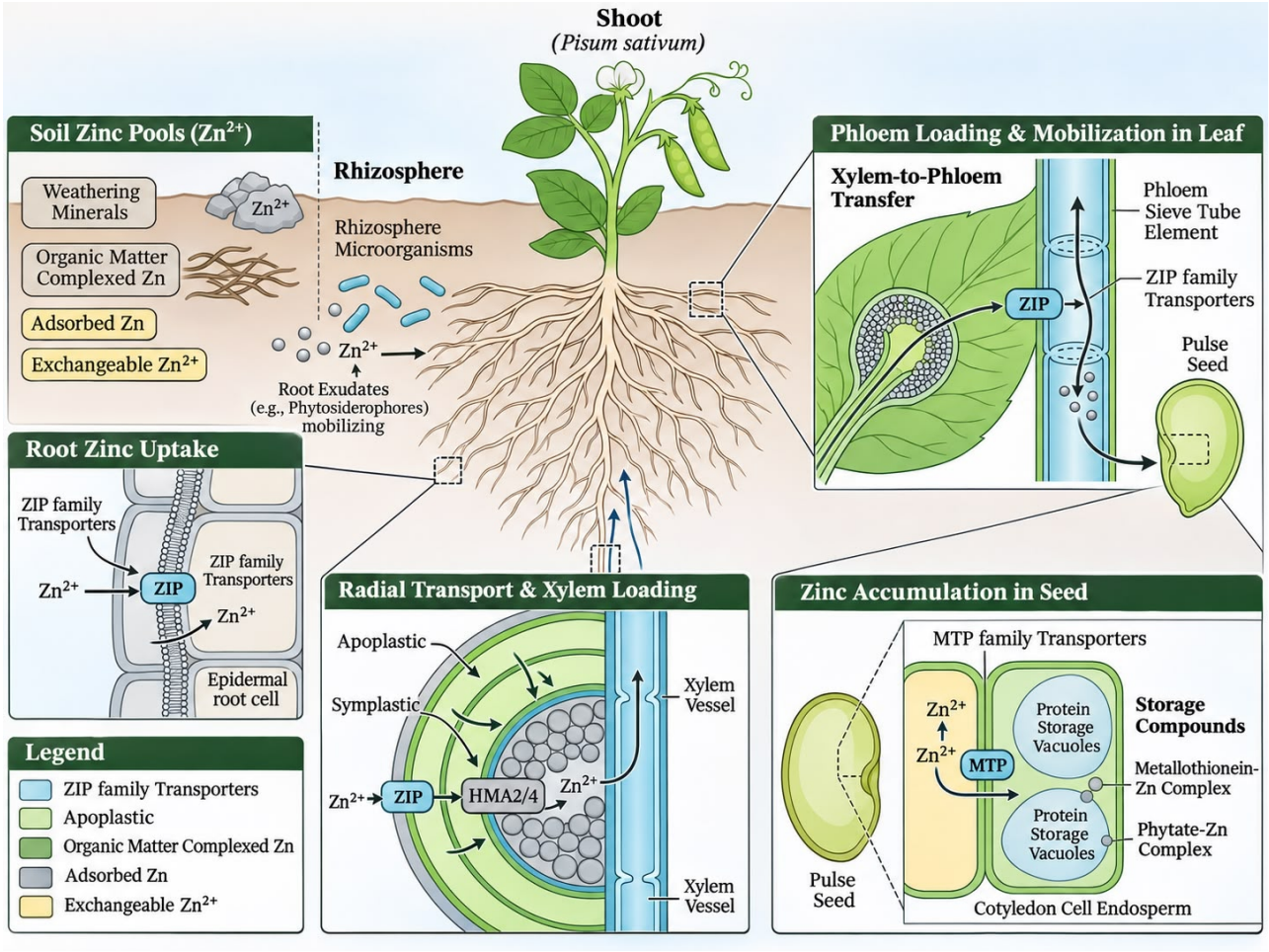


Fig.1. Zinc Uptake, Transport and Accumulation Mechanism in Pulse Crops

2.1 Soil Zn Availability and Root Uptake

Agricultural soils have good total zinc, but only a small amount is available in a soluble form to the roots. Zinc availability in soil is often reduced under high pH and calcareous conditions. Excessive application of phosphorus fertilizers can further limit zinc uptake by plants, causing zinc deficiency even when the total zinc content in the soil is adequate. However, plants can actively improve zinc acquisition through root exudation. Roots release organic acids and chelating compounds that help mobilize zinc from soil particles. This process is further supported by zinc-solubilizing microorganisms in the rhizosphere, which increase the availability of zinc for plant uptake. Varieties respond differently to the same fertilization treatments due to the significant differences in the ability to take up the fertilizer under zinc limiting conditions, which is due to different genotypes in ability to uptake these fertilizers through stronger root systems or more active transporters. The effectiveness of zinc management depends on the interaction between soil conditions, plant genotype, and rhizosphere processes. Therefore, zinc application strategies should be tailored to specific environments rather than following a general recommendation. Site-specific nutrient management can improve zinc use efficiency and provide better outcomes compared with uniform zinc fertilization practices (Upadhayay et al., 2025; Pradhan et al., 2025).

2.2 Molecular Regulation of Zinc Transport

In higher plants, zinc is regulated in flow, moving it and partitioning it without causing toxicity, at the plant level, there are several families of transporter involved. Regulation of zinc has been studied chiefly in the model species and cereal plants. In rice, *OsZIP* family members play a role in zinc uptake and redistribution, and *OsHMA2* has been found to be involved in long distance zinc transport to developing grains. In barley, the *HvZIP7* transporter contributes to zinc homeostasis by regulating zinc distribution within plant tissues. Likewise, metal tolerance proteins and vacuolar transport systems found in model and cereal species, offer valuable information on zinc

storage and distribution. These mechanisms are not expected to be the same in pulses. However, because the activity of transporters, gene expression and seed loading mechanisms are not as well validated in these crop types, and because the xylem loading pathway and redistribution to tissues is not as well understood as in maize, determining the amount of zinc that is translocated to the seed versus being held in the roots and leaves. Metallothionein and Phytochelatins are metal binding peptides that help regulate zinc levels within plant cells. They bind excess zinc to prevent toxicity while maintaining an adequate amount of available zinc for essential metabolic processes. Although many genes have been discovered to play a role in these processes, the majority have been identified under controlled conditions, and expression may vary significantly as a function of soil type, climate and management. This is only the first half of the challenge; validation of the behavior of these genes in the field and relating this information to breeding and agronomic decisions is the second half (Shahzad et al., 2021; Kaushal et al., 2024).

2.3 Seed Zinc Loading Mechanism and Bioavailability

The most critical and last step is xylem to phloem transfer and zinc specific transporters from vegetative tissue to seeds, as pulses are consumed as seeds. During seed filling, zinc is mainly stored in association with proteins, amino acids, and phytate. The nutritional value of zinc depends not only on its total concentration but also on its chemical form and bioavailability. There is a lot of phytic acid in the pulse seeds, which is a strong chelator of zinc and reduces the absorption of zinc in the human gut, so the amount of zinc that may be listed in the table can be high even though very little is available after cooking and absorption. To maximize the benefits of zinc biofortification, improving zinc bioavailability is essential. This can be achieved by using molecular approaches to regulate zinc storage in seeds or by developing crop varieties that combine high zinc content with reduced phytate levels. Seed loading is the point where plant physiology meets human nutrition and is the phase that determines whether genetic and agronomic improvements will be realized in the human diet (Zhang et al., 2021; Lakshmanan et al., 2025).

III. GENETIC IMPROVEMENT STRATEGIES FOR ZINC BIOFORTIFICATION IN PULSE CROPS

Genetic improvement is the most durable route to zinc biofortification because it does not depend on repeated inputs, but zinc accumulation is a quantitative trait shaped by many interacting genes and by the environment. Breeding strategies for zinc biofortification require a broader approach than conventional breeding focused only on yield or disease resistance. Successful varieties should combine improved mineral concentration with high productivity, environmental adaptability, and consumer acceptance. The use of classical breeding in combination with molecular tools, high throughput phenotyping and genomics is now the norm to accelerate this multi-trait selection (Tessema et al., 2026; Singh et al., 2016).

3.1 Conventional Breeding Approaches

Conventional breeding utilizes the large naturally occurring genetic diversity for zinc content within chickpea, common bean, pea and lentil germplasm, including landraces and wild relatives. These collections have been screened and a number of zinc enriched genotypes have been repeatedly found which have been used as parents in improvement program. However, zinc content by itself is not enough for a variety to be useful, some zinc enriched varieties have low yields and poor plant architecture, or they are not well adapted to the local environment, so the decision of which varieties to use must be made based on zinc levels as well as agronomic characteristics. The most widely used method for screening zinc concentration is laboratory based mineral analysis. Although this approach provides accurate measurements, it is costly and time consuming when applied to large germplasm collections. Therefore, it`s use is often limited in large scale breeding programs. Conventional breeding can contribute to sustained nutritional improvement and may reduce reliance on supplementation-based strategies, while the polygenicity of zinc accumulation and its high environmental sensitivity are being improved by molecular breeding and genomics (Rani et al., 2025; Kaur et al., 2020).

3.2 Molecular Breeding and Marker-Assisted Selection

Using molecular tools, breeders can now monitor the genomic regions associated with zinc accumulation without having to wait for seeds to mature and then chemically analyze them. Markers for zinc concentration have been identified and mapped to genomic regions known as Quantitative Trait Loci (QTL) to enable early selection of promising seedlings by Marker -Assisted Selection (MAS) significantly reducing breeding cycles. Genomic selection takes this one step further by combining genome wide marker data with prediction of breeding value for a trait influenced by numerous small effect genes, a model that is more appropriate for zinc accumulation than a single-marker approach. These approaches can significantly accelerate the development of zinc-enriched crop varieties. However, many identified QTLs and molecular markers show strong environmental dependence and may not

perform consistently across different locations. In addition, the final zinc concentration in seeds is influenced not only by genetic potential but also by soil conditions and crop management practices. The next step in molecular breeding is not to find additional zinc genes but to combine genomic selection with precision agronomic data to ensure that desirable genotypes are used in the setting where they are likely to be used (Garcia-Oliveira et al., 2024; Tessema et al., 2026).

3.3 High Throughput Phenotyping (HTP) and Genomic Tools

High Throughput Phenotyping (HTP) imaging, spectroscopy and automated sensing are therefore important to assess the mineral acquisition capacity and the root architecture, in addition to seed concentration, as these traits are related to Zinc. HTP in combination with genomic tools like genome wide association studies, transcriptomics, and metabolomics can help to explain, not only which genotypes accumulate more zinc, but why, as it relates to underlying regulatory pathways. Precision breeding relies on the integration of genomic information, high-throughput phenotyping, and environmental data. However, pulse crops still lag behind cereals in terms of available reference genomes and advanced phenotyping resources. Future breeding systems will need to overcome the challenge of converting molecular predictions into consistent field performance by combining these approaches effectively (Madurapperumage et al., 2025; Garcia-Oliveira et al., 2024)

3.4 Challenges in Genetic Improvement

However, there are a number of practical constraints to the extent to which genetic improvement can be achieved alone. The selection of zinc concentration may come at the expense of yield as mineral accumulation will compete with biomass and grain filling processes, with the risk of obtaining agronomically less attractive varieties. The second important limitation is the genotype × environment interaction: the same variety may be the most suitable in one environment, but not in another, casting doubt on the existence of a universally best variety. Zinc accumulation in seeds is a complex polygenic trait that involves multiple processes, including zinc uptake, transport, redistribution, and seed loading. Improving zinc concentration alone does not always result in better nutritional outcomes because factors such as phytate and other antinutrients can reduce zinc bioavailability. Breeders also have a variety of genomic resources and transformation systems to work with for pulse crops, This approach should not be viewed as a universal solution to zinc deficiency. Rather, it should be integrated into a broader strategy for improving crop nutrition and productivity, alongside precision agronomic practices and microbial technologies (Tessema et al., 2026; Shahzad et al., 2021).

IV. PRECISION AGRONOMIC APPROACHES FOR ZINC BIOFORTIFICATION IN PULSE CROPS

Site-specific agronomic management is essential to accessing the genetic potential. Precision agronomy aims to match the fertilizer application method, timing, and dose to soil chemistry and genotype; rather than being applied uniformly, zinc fertilization is aimed to ensure uptake of the applied fertilizer is not greater than what can be used by the plants. This issue is particularly important for pulse crops because many species have a high zinc requirement and are often grown in zinc-deficient soils. These crops are frequently cultivated in regions where human zinc deficiency is also widespread. Agronomic practices therefore play a key role in connecting the genetic potential of crops with practical improvements in zinc nutrition at the field level. The expression of genetic potential for zinc accumulation is strongly influenced by environmental conditions and crop management practices. **Figure 2** presents the genotype × environment × management (G × E × M) framework, illustrating how these factors interact to determine zinc availability, uptake efficiency, and seed enrichment. This integrated perspective helps explain why similar zinc management practices may produce different outcomes across soils, environments, and pulse genotypes (Shivay et al., 2016; Hasanuzzaman et al., 2023).

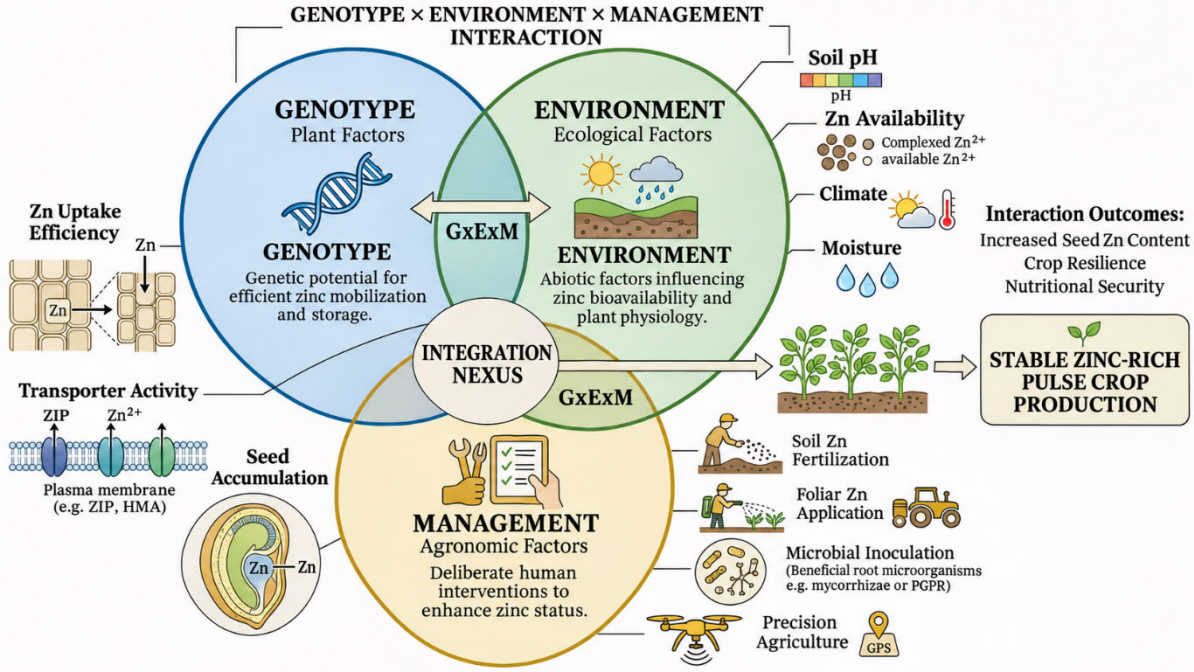


Fig.2. Precision Agronomy Framework: Genotype × Environment × Management Interaction

4.1 Soil Zn Fertilization

The most traditional agronomic solution is the application of zinc sulfate, zinc oxide or chelated formulations, achieving the effect by simply increasing the amount of plant available zinc in the root zone. However, the effectiveness of zinc fertilization largely depends on soil chemical properties. Alkaline and calcareous soils can reduce zinc availability by increasing zinc adsorption and precipitation. In addition, high phosphorus levels may promote the formation of insoluble zinc–phosphate compounds, which limits zinc uptake and reduces fertilizer efficiency. Plants can partially overcome these limitations through the action of root exudates and rhizosphere microorganisms, which help release and mobilize zinc from soil and different genotypes can use the zinc available to them more or less efficiently, so it is not always possible to achieve the same results in terms of growth with the same rates of fertilizer among varieties. This variability argues against a uniform zinc-fertilization strategy and using genetically efficient varieties should be the main approach to zinc fertilization with soil application being a secondary measure (Shivay et al., 2016; Baghaie & Khosravi-Dehkordi, 2025).

4.2 Foliar Zn Application

The alternative application of zinc overcomes soil chemistry issues that impede root uptake, providing zinc to leaf surfaces where fixation is not a problem. The timing is the most important here; applications made at flowering or seed filling, when nutrient demand and delivery to the developing seeds is highest, are much more effective at delivering zinc to grain than earlier applications. It is particularly effective in high pH soils or zinc immobilizing soils, but is influenced by leaf characteristics, rainfall, temperature and formulation and has to be repeated to increase labor and costs. Foliar application is best combined with soil management and zinc efficient genotypes, as it is a treatment for a symptom, and not the soil constraint (Shivay et al., 2016).

4.3 Nano-Zn Fertilizers & Advanced Delivery Systems

Given that the delivery of zinc in plants may be influenced by the small particle size and high surface area, and by changing the dissolution behavior of nano-sized zinc, it has been suggested that Nano-Zn fertilizers could be used as an alternative to the delivery strategy. The potential for the improvement of Zn-use efficiency in plants using nanoparticles could be due to a change in the rate of dissolution, nanoparticle-based zinc fertilizers reduce immediate fixation reactions and provide a slower, more controlled release of zinc compared with conventional fertilizer sources. But how pronounced are these effects, and whether they are an actual advantage of the nanoscale is unclear (Upadhayay et al., 2025; Pradhan et al., 2025). One of the big unanswered questions is if Nano-Zn offers benefits other than what soluble zinc sources can do. Zinc nanoparticles could also dissolve in many soil environments, thereby releasing zinc ions (Zn^{2+}); the latter is the most important chemical form that plants can absorb by their roots. Therefore, the improvement in plant zinc

uptake is mainly associated with the increased availability of Zn^{2+} ions rather than any specific DNA-binding mechanism of the nanoparticles. It is important to differentiate between the effects of the nanoparticles and just Zn ions being released in order to accurately examine the agronomic value of Nano-Zn fertilizers. Evidence of current Nano-Zn application in crop biofortification is context specific and depends on the features of the Nano-Zn, soil properties, the rate of application, the crop species, and the environment. The availability or uptake of zinc usually increases in laboratory and controlled-environment tests but is not well demonstrated on a field scale. The following issues are also areas that need further investigation: possible persistence of nanoparticles in soil, the possibility that nanoparticles can transform in soil, possible interaction with the microbial community, possible environmental accumulation, reduced production cost, and regulatory assessment (Upadhayay et al., 2025). Thus, Nano-Zn represents a promising approach for improving zinc delivery; however, its advantages over conventional zinc fertilizers require further empirical validation. The real impact on pulse zinc biofortification will rely on the ability to show consistent field benefits, the understanding of whether the benefits are from the nano-specific properties or Zn^{2+} release, and the need to integrate its application with zinc-efficient genotypes, improved soil management practices, and biological approaches (Pradhan et al., 2025).

4.4 Interaction Between Genotype × Environment × Management

A point not always emphasized in specific studies underlying all of the above agronomic discussion is that the genotype x environment x management interaction must be considered, and that zinc accumulation is not predictable based on any single factor. The biological celling is set by genotype, how much of that is realized depends on environment and management determines the level of seed zinc yield. Breeding studies that do not account for relevant management condition may provide an incomplete assessment of zinc biofortification potential as is an agronomic trial without consideration of genotype. The explicit acknowledgment of this genotype × environment × management interaction is what makes it possible to integrate genetic, agronomic, and biological aspects of this review into a unified framework, rather than treated as parallel and disconnected literatures (Tessema et al., 2026).

V. BIOTECHNOLOGICAL INNOVATIONS IN ZINC BIOFORTIFICATION

Breeding and agronomy technologies can only address the physiological aspects of zinc biofortification, while Biotechnology can provide a mechanistic level that can identify and in some cases directly manipulate the molecular pathways which regulate zinc uptake, transport, and storage. Zinc accumulation in pulses is regulated by multiple genetic pathways and is strongly influenced by environmental conditions. Advances in genome editing and omics-based approaches are helping researchers identify the key regulatory genes involved in zinc uptake, transport, and accumulation. Their real value, however lies in their integration with breeding and proven agronomic performance in the field (Kaushal et al., 2024; Venkataraman et al., 2025).

5.1 CRISPR/Cas Genome Editing

All the genes that play a role in the transport, storage and loading of zinc in the seed could be potential targets for genome editing, but the quality of evidence varies significantly among crops. Current knowledge of zinc transporter targets is largely derived from studies in cereal crops and model species, while information from pulse crops remains comparatively limited. Examples include *OsZIP* family members and *OsHMA2* in rice, as well as *HvZIP7* in barley, which are functional evidence of zinc uptake and grain-directed transport, respectively, in cereals. These are genes that could be mechanism targets in pulse improvement, but are not directly validated (Wang et al., 2025; Shahzad et al., 2021).

For pulse crops, there is evidence of homologous transporters families identified by genome sequencing and comparative analysis, for example ZIP and HMA related genes in *P. vulgaris* and chickpea. yet, there is limited evidence for the functional validation of individual pulse orthologues and their ability to increase zinc uptake, transport or seed accumulation. Hence, selection of candidates for editing of the pulse genome is still largely based on comparative genomics and expression-based evidence and not on evidence of gene-to-trait relationship as has been done in rice and other cereals (Kaushal et al., 2024).

The ones that affect bioavailability may be edited to increase the concentration of zinc or increase its nutritional accessibility in principle. However, zinc homeostasis is controlled by a complex network of interacting pathways. Therefore, modifying a single gene may produce unintended effects on other minerals that share similar transport systems. In addition, practical application of these approaches is limited by regulatory challenges and difficulties in transforming some pulse crops. It is important to realize that CRISPR is not a replacement for breeding but rather an extra tool added to breeding, and large-scale application will only be considered after identifying genetic backgrounds with strong potential, and then validated using physiology and field testing. Although molecular targets

have been identified, no commercial pulse cultivar has been released nor has any agronomically validated pulse cultivar have been developed through the editing of zinc transporter genes.

5.2 Transcriptomics and Metabolomics Approaches

Transcriptomic and metabolomic analyses provide valuable insights into potential targets for genome editing by revealing the molecular mechanisms involved in zinc regulation. These approaches have identified genes that are activated under zinc deficiency, adequate zinc supply, and zinc enrichment conditions. The identified genes include zinc transporters, regulatory proteins, and stress-responsive pathways, which help explain the variation in zinc accumulation among different genotypes. The biochemical side like organic acids and chelating compounds that mobilize zinc in the rhizosphere, with metal binding metabolites that control its storage and safe handling inside the plant are added by metabolomics, suggesting better bioavailability than just increasing the amount present. The integration of these omics layers with genomics provides a systems view of zinc regulation, rather than a gene by gene view, which is useful for genomic selection and editing. The challenge is that most of this work is conducted in controlled environments that to a certain extent, do not replicate soil, climate and management variability in nature and the next step is to complement omics with field phenotyping and environmental data sets (Kaushal et al., 2024).

5.3 Regulation of Zn accumulation at the genetic level

Synthesizing the molecular mechanism, ZIP transporters play a dominant role in the uptake of other plant tissues, as their expression is induced under zinc deficiency, while HMAs and MTPs are responsible for distributing zinc among the subcellular locations and to the developing seeds, and MTs and PCs act as buffers to accumulate excess zinc in a minimal form, maintaining some zinc in available form for metabolism. These steps are interdependent, so an increase in expression of one of the genes in the chain will not necessarily increase the zinc content of the seeds if another step becomes limiting. This calls for the regulation networks not the single gene to be targeted, which is the same systems level approach used with the integrated framework that combines information from expression studies, metabolomics, genomic selection, and physiological data to know where to look for the major challenges (Tessema et al., 2026).

5.4 Current Limitations

Some limitation restricts the application of biotechnology for pulse zinc biofortification: there remains a significant disconnect between lab and field performance; regulation of single gene biofortified crops varies greatly across regions and pulses are complex crops with complex regulation of zinc. In some countries, genome edited plants are subject to different rules depending on whether or not the genome edited plant contains foreign DNA. The regulatory status of transgene-free genome-edited plants varies among countries. In some regions, these plants are regulated similarly to conventionally bred crops. However, other regulatory systems classify them under broader GMO frameworks, requiring additional assessment, documentation, and approval processes. These differences influence the rate of progress, cost and availability of deploying genome-edited biofortified crops, especially pulse crops with limited transformation and regeneration systems, and comparatively low genetic resources and transformation systems in the pulse crops compared to major cereals. None of this makes biotechnology unnecessary, but it does indicate that the value of biotechnology will be primarily as a specific contributor to the breeding and agronomy programs providing validated molecular targets and understanding (Venkataraman et al., 2025).

VI. MICROBIAL ASSISTED ZINC BIOFORTIFICATION

Rhizosphere biology is necessary to first make soil zinc available for well developing genotypes and well managed fertilizer programs to be effective. Root-associated microorganisms, such as zinc solubilizing bacteria, plant growth promoting rhizobacteria, and arbuscular mycorrhizal fungi, help to mobilize fixed zinc into bioavailable pools, increase root growth and enhance nutrient uptake and stress resistance. The effectiveness of microbial tools relies heavily on plant genotype, soil conditions and management and therefore are best considered a biological complement to both breeding and agronomy and not a replacement for either (Upadhayay et al., 2025; Sultan et al., 2025).

6.1 Zinc Solubilizing Bacteria (ZSB)

The primary drawback of soil-based biofortification is that the bulk of zinc, whether added or present in the soil, is unavailable to the roots, and these ZSB offers a strategic solution to this challenge. Microorganisms capable of mobilizing these zinc forms mainly produce organic acids, such as gluconic acid and citric acid. These acids reduce the pH of the rhizosphere, which helps release zinc ions from soil minerals. In addition, these microorganisms produce chelating compounds that bind zinc and reduce its reattachment to soil particles, thereby improving zinc availability for plants. While the practical interest is

obvious, better zinc use efficiency and fertilizer independence especially important in low input systems, the success the strain in the lab does not guaranty field efficacy because the performance in the field is influenced by soil pH, soil temperature, soil moisture, and competition from indigenous soil microorganisms. Although microbial inoculants have potential as a practical approach, their effectiveness may vary across different crops and soil environments. Future progress may depend more on the development of crop-specific microbial consortia, combined with the identification of zinc-efficient plant genotypes and suitable soil conditions. Such integrated strategies could provide more reliable solutions for improving zinc uptake and utilization in crops. These may be especially useful in low input systems where fertilizer costs, fixation of zinc and Zinc use efficiency is a limiting factor in the effectiveness of conventional zinc application. While chemical zinc fertilization has been a common practice, the application rate is suggested to be around 5 kg Zn ha^{-1}, and the availability of the added zinc can be low due to high cost of the chemical fertilizers and because a large fraction of the applied zinc can become inactive in the soil. This constraint is an indication of the potential benefit of ZSB to raise soil zinc availability through the mobilization of soil zinc pools that are not readily accessible to plants, and to improve zinc use efficiency, especially in low-input farming systems (Upadhayay et al., 2025; Pradhan et al., 2025).

6.2 Plant Growth Retarding Rhizobacteria (PGRB)

Beyond solubilization, the beneficial effects of PGPR are greater, principally as a result of their production of phytohormones, including indole-3-acetic acid (IAA), which increases the root surface area and provides access to a greater soil volume, a worthwhile benefit for zinc which is poorly mobile in soil. Some strains mobilize zinc itself, others help to increase the uptake of phosphorus or all other nutrients, and many help to increase drought and salt and temperature stress tolerance, which would otherwise reduce nutrient uptake. The number of PGPR studies focusing on pulse crops that measure seed zinc concentration or bioavailability is relatively low, so there is a disconnect between the known plant-growth potential and the known nutritional benefits. Studies on cereal crops have shown that microbial interventions can increase seed zinc concentration and improve related nutritional traits. However, these outcomes have not been extensively investigated in pulse crops. Further research is needed to explore how microbial based approaches can enhance zinc biofortification and nutritional quality in pulses. Increased evaluations of pulse-specific parameters, such as seed zinc concentration, zinc partitioning, and zinc bioavailability measurement will thus be vital to assessing the real contribution of PGPR-based approaches toward human nutrition. The effectiveness of PGPR, similar to ZSB, may be improved when it is integrated with zinc-efficient plant genotypes and precision fertilization strategies. Rather than relying on microbial inoculation alone, a combined approach could provide a more sustainable and reliable method for enhancing zinc availability and uptake in crops (Sani & Yong, 2022; Tariq et al., 2025).

6.3 Arbuscular Mycorrhizal Fungi

A poorly mobile nutrient, such as zinc, can be particularly beneficial as arbuscular mycorrhizal (AM) fungi increase the effective root volume beyond the reach of the roots. In addition to nutrient absorption, these associations help maintain soil structure and stress tolerance, which indirectly aids zinc uptake in drought and/or salinity conditions. Their advantage is variance in fungal species, host genotype and soil conditions and in some cases controlling trials do not produce the same results as in the field, therefore the selection of plant fungus combinations is important. Like the other microbial solutions, this will become a dependable biofortification solution, not inconsistence, if used in combination with efficient microbial populations, or zinc efficient varieties and strategic nutrient management (Berruti et al., 2016; Shree et al., 2022).

VII. INTEGRATED BIOFORTIFICATION FRAMEWORK: FROM GENES TO FIELDS

Each of these areas of genetic improvement, precision agronomy, biotechnology and microbial technologies has made real advances but alone they leave important gaps: a zinc efficient genotype may not perform well if the soil is not of high quality; the optimal use of fertilizer can be ineffective on a poorly suited variety; and molecular discoveries can remain untested in the field. The framework presented here takes the inclusive approach of genotype × environment (G × E) thinking, but includes management (M) as a design variable that affects the expression of genetic potential under field conditions. This approach takes a new view of agronomic interventions, such as fertilizer application and microbial inoculants, as factors that can be optimized simultaneously with genotype and environmental factors, rather than as external treatments applied following genotype selection. The approach involves combining field management practices with breeding to maximize the probability of achieving stable zinc accumulation in pulse crops, creating an approach to breeding that is linked to field management. Precision agronomy provides soil and nutrient conditions for the biological capacity to accumulate zinc to be expressed, while microbial technologies can improve zinc availability

in the rhizosphere, making zinc more accessible for plant uptake. However, the effectiveness of these approaches also depends on understanding the molecular mechanisms that regulate zinc mobilization, transport, and utilization within plants that will be provided by biotechnology will allow all three to be targeted more precisely. The main contribution of this model is not simply the inclusion of genotype × environment (G × E) interactions, as these concepts are already well established in crop science. Instead, the model emphasizes the importance of integrating management practices and biological enhancement strategies into the decision-making process. The framework captures genotype and environment, as well as management interventions like zinc application and microbial inoculation, in order to help establish a pathway to linking molecular discoveries and breeding decisions with field-level biofortification results in farmer`s fields. Integrating these individual components into a single framework provides a pathway for translating molecular discoveries into field-level outcomes. **Figure 3** illustrates the proposed gene-to-field biofortification pipeline, where genetic improvement, precision agronomy, microbial technologies, and genome-based approaches operate together within a G × E × M framework. This model emphasizes that sustainable zinc enrichment requires coordinated optimization of biological potential, environmental conditions, and management strategies rather than reliance on a single intervention (Tessema et al., 2026; Lakshmanan et al., 2025).

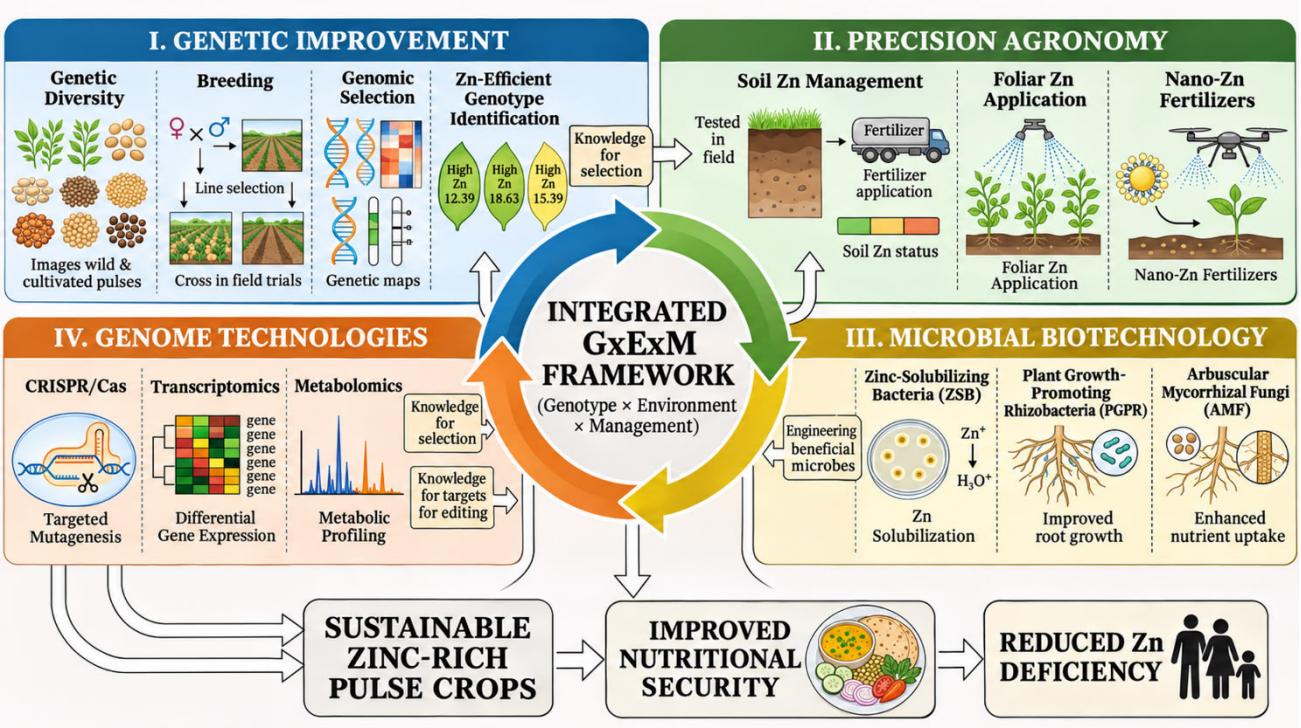


Fig.3. Integrated Biofortification Pipeline: From Genes to Fields

To further clarify the contribution of each component within the integrated framework, **Table 1** summarizes the major strategies involved in zinc biofortification, their functional roles, current limitations, and future research directions. This comparison highlights the need to combine genetic improvement, precision management, microbial enhancement, and molecular approaches to develop stable and sustainable zinc-rich pulse production systems.

Table 1. Integrated Framework for Sustainable Zinc Biofortification in Pulse Crops: From Genes to Fields

Integrated Component	Key Approaches	Main Function in Zn Biofortification	Current Challenges	Future Integration Direction	Representative Evidence
Genetic Improvement	Conventional breeding, germplasm screening, QTL mapping, marker assisted selection, genomic selection	Develop Zn efficient pulse genotypes with improved Zn uptake, transport, and seed accumulation capacity	Complex inheritance of Zn traits, genotype × environment interaction, yield–Zn tradeoff	Combine genomic information with environmental data to develop stable Zn enriched varieties	Rani et al. (2025); Garcia-Oliveira et al. (2024); Madurapperumage et al. (2025)
Precision Agronomy	Soil Zn fertilization, foliar Zn application, site-specific nutrient management, advanced Zn delivery systems	Enhance Zn availability and improve expression of genetic potential under field conditions	Soil variability, Zn fixation, inconsistent fertilizer response	Develop genotype-specific Zn management strategies based on soil and crop requirements	Shivay et al. (2016); Upadhayay et al. (2025); Pradhan et al. (2025)
Microbial Biotechnology	Zinc solubilizing bacteria, PGPR, arbuscular mycorrhizal fungi	Improve rhizosphere Zn mobilization, root development, and nutrient acquisition efficiency	Microbial survival, environmental dependency, field level inconsistency	Design crop specific microbial consortia compatible with Zn efficient genotypes	Upadhayay et al. (2025); Tariq et al. (2025); Shree et al. (2022)
Genome Technologies	CRISPR/Cas editing, transcriptomics, metabolomics, functional genomics	Identify and modify Zn regulatory pathways controlling uptake, transport, and storage	Complex molecular networks, regulatory barriers, limited pulse transformation systems	Apply multi omics and genome editing to accelerate Zn trait improvement	Shahzad et al. (2021); Kaushal et al. (2024); Wang et al. (2025); Venkataraman et al. (2025)
G × E × M Integration *(Core Novelty)*	Genotype selection + environmental characterization + optimized management	Connect genetic potential with real field performance for stable Zn enrichment	Most studies evaluate genotype, environment, and management separately	Develop predictive biofortification systems using AI, modelling, and precision agriculture	Tessema et al. (2026); Hasanuzzaman et al. (2023); Zhang et al. (2025)
Final Outcome	Integrated genes to fields approach	Sustainable production of Zn enriched pulse crops with improved nutritional quality	Requires multidisciplinary collaboration and field validation	Transition from individual approaches to complete biofortification pipelines	Lakshmanan et al. (2025); Tessema et al. (2026); Spain et al. (2026)

VIII. CHALLENGES AND FUTURE PERSPECTIVES

Despite this integrated perspective, there are formidable scientific and agricultural challenges from concept to the broad adoption of improved varieties, from accumulation mechanisms to economics.

8.1 Scientific Challenges

A persistent science challenge is the integration of zinc concentration with yield stability and stress tolerance under

breeding programs, which requires multi trait selection (combining zinc density with yield stability and stress tolerance through genomic selection and phenotyping) instead of selection based on zinc concentration alone. A second major challenge is improving zinc bioavailability. Increasing the total zinc concentration alone does not always result in better nutritional outcomes, as zinc absorption can remain limited when phytic acid and other antinutritional compounds bind zinc during cooking and digestion. This is why it is important to consider nutritional effectiveness as a composite trait, which requires an integrated approach to breeding and molecular studies of transport, storage, and antinutrient pathways. A third challenge is strong genotype × environment interaction: a line's zinc performance in one location does not necessarily reflect its performance in another location, and agronomic or microbial interventions are similarly sensitive to environment, raising the need to combine environmental and soil data with genetic and phenotypic data to provide location specific and climate resilient recommendations rather than broadly adapted cultivars (Tessema et al., 2026; Zhang et al., 2025).

8.2 Agricultural Challenges

Not every successful scientific innovation is used into widespread adoption. Farmers' choices of crop varieties are influenced by multiple factors, including yield potential, disease resistance, maturity period, market demand, and production cost. Therefore, biofortified pulses must demonstrate both nutritional benefits and strong agronomic performance to achieve broader acceptance. Participatory breeding approaches, farmer engagement, and awareness programs are also essential to promote the adoption of micronutrient-rich crops. A second obstacle is cost and infrastructure: all four technologies—genomic selection, genome editing, precision agriculture, and microbiome technologies require technical expertise and investment, which are most lacking where zinc deficiency is severe and smallholder farms dominate. This will require the ability to present low-cost diagnostics, locally adapted microbial products, and practical and simplified nutrient-management guidance to farming systems that can benefit from the most advanced science (Spain et al., 2026; Lakshmanan et al., 2025).

8.3 Future Research Directions

Genomic, phenotypic, environmental and management data integration will be revolutionized by the use of AI and machine learning to predict zinc efficient genotypes and aid in breeding decisions much more quickly than current selection methods. In parallel, precision agriculture tools, including digital soil mapping, remote sensing, and predictive nutrient models, can shift the timing of the application of zinc from general recommendations to site and genotype-specific applications. There are additional opportunities to consider in addition to single bacterial or fungal strains: the ability to create beneficial microbial communities that can be customized for specific soil and genotype could provide a more sustainable and powerful pathway to better zinc uptake without the need for chemical inputs. All this is made even more necessary by climate change, because drought, heat and changing soil conditions will change zinc availability and uptake in plants, making stress tolerance an ever-important secondary trait to zinc efficiency. Progress in this area relies more on integrating genetic improvement, precision agronomy, microbial enhancement and biotechnology into a coherent-genes to fields pipeline than on any one new technology that could help achieve this goal, and is most likely to result in the production of consistently zinc enriched, zinc productive and zinc adapted pulse crops that farmers are facing. (Garcia-Oliveira et al., 2024; Madurapperumage et al., 2025)

IX. CONCLUSION

Zinc biofortification of pulse crops represents a sustainable strategy to address micronutrient deficiency and improve nutritional security. However, zinc accumulation is a complex trait controlled by multiple interacting factors, including genetic background, soil conditions, environmental variability, and crop management practices. Therefore, achieving consistent zinc enrichment requires more than a single intervention and demands an integrated approach. Genetic improvement provides the foundation for developing zinc-efficient pulse varieties, while precision agronomy enhances zinc availability and supports better expression of genetic potential under field conditions. Microbial-based technologies, including zinc-solubilizing bacteria, plant growth-promoting rhizobacteria, and mycorrhizal fungi, further improve rhizosphere processes and nutrient acquisition. In parallel, biotechnology approaches such as genome editing, transcriptomics, and metabolomics provide valuable insights into zinc uptake, transport, and storage mechanisms. Despite considerable progress, major challenges remain, including genotype × environment interactions, limited field validation, variable performance of microbial inoculants, and difficulties in large-scale adoption. Addressing these limitations requires stronger integration among genetic resources, environmental data, precision management, and biological innovations. The proposed gene-to-field framework provides a pathway to connect molecular discoveries with practical agricultural applications by optimizing genotype, environment, and management interactions. Future research should focus on multidisciplinary approaches, including

multi-omics integration, precision agriculture, crop-specific microbial solutions, and field-based validation. Such coordinated efforts can accelerate the development of stable zinc-rich pulse crops, enhance food quality, and contribute to long-term solutions for global nutritional security.

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REFERENCES

[1] H. Baghaie and A. Khosravi-Dehkordi, "The Critical Role of Zinc in Improving Public Health and Crop Quality: Examining Challenges and Strategies for Soil Enrichment in Developing Countries," Int. J. Environ. Health Eng., vol. 14, no. 4, pp. 1–10, 2025, doi: 10.4103/ijehe.ijehe_62_24.

[2] L. Garcia-Oliveira et al., "The importance of genotyping within the climate-smart plant breeding value chain – integrative tools for genetic enhancement programs," Front. Plant Sci., vol. 15, no. February, pp. 1–26, 2024, doi: 10.3389/fpls.2024.1518123.

[3] M. Tessema et al., "Crop biofortification for enhanced nutritional density: Breeding, agronomical, and biotechnological strategies," Crop Sci., vol. 66, no. 1, pp. 1–22, 2026, doi: 10.1002/csc2.70246.

[4] Madurapperumage, M. Z. Naser, L. Boatwright, W. Bridges, G. Vandemark, and D. Thavarajah, "High-throughput phenotyping platforms for pulse crop biofortification," Plants People Planet, vol. 7, no. 1, pp. 49–61, 2025, doi: 10.1002/ppp3.10568.

[5] Shree, U. Jayakrishnan, and S. Bhushan, "Impact of key parameters involved with plant-microbe interaction in context to global climate change," Front. Microbiol., vol. 13, no. September, pp. 1–18, 2022, doi: 10.3389/fmicb.2022.1008451.

[6] Kaushal et al., "Transcriptional engineering for value enhancement of oilseed crops: a forward perspective," Front. Genome Ed., vol. 6, no. January, pp. 1–20, 2024, doi: 10.3389/fgeed.2024.1488024.

[7] H. M. Sultan, M. A. Raza, M. Fatima, and T. Sajid, "Turkish Journal of Agriculture - Food Science and Technology Innovative Approaches to Rhizosphere Engineering with Plant Growth Promoting Microorganisms in Agricultural Practices," vol. 13, no. 5, pp. 1324–1343, 2025.

[8] H. Tariq, S. Subramanian, A. Geitmann, and D. L. Smith, "Bacillus and Paenibacillus as plant growth-promoting bacteria in soybean and cannabis," Front. Plant Sci., vol. 16, no. June, pp. 1–14, 2025, doi: 10.3389/fpls.2025.1529859.

[9] H. Zhang, H. Sun, Z. Tang, and G. Wang, "Integrated agronomy of pea (Pisum sativum L.): a review on cultivation, harvesting, and storage for sustainable agriculture," Front. Plant Sci., vol. 16, no. September, pp. 1–18, 2025, doi: 10.3389/fpls.2025.1670445.

[10] J. M. Kafer et al., "Metabolic engineering of soybean for improving grain quality for animal consumption," Front. Plant Sci., vol. 17, no. February, pp. 1–16, 2026, doi: 10.3389/fpls.2026.1738805.

[11] J. Mbiu, T. Assefa, C. Mukankusi, J. C. Rubyogo, and M. Philipo, "Consumer traits of common beans: a global and regional perspective on seed coat darkening, cooking time, protein, and mineral content," Front. Nutr., vol. 12, no. September, 2025, doi: 10.3389/fnut.2025.1658338.

[12] J. Rani, D. Beniwal, S. S. Dhull, V. Gulia, P. Sangwan, and G. Abdi, "Biofortification of Cicer arietinum to enhance nutrient-rich diets in semi-arid regions," Discov. Sustain., vol. 6, no. 1, 2025, doi: 10.1007/s43621-025-01554-6.

[13] M. Hasanuzzaman, M. S. Tahir, M. Tanveer, and A. N. Shah, Mineral Biofortification in Crop Plants for Ensuring Food Security. 2023. doi: 10.1007/978-981-99-4090-5.

[14] M. N. H. Sani and J. W. H. Yong, "Harnessing Synergistic Biostimulatory Processes: A Plausible Approach for Enhanced Crop Growth and Resilience in Organic Farming," Biology (Basel)., vol. 11, no. 1, 2022, doi: 10.3390/biology11010041.

[15] N. Pradhan et al., "A review on microbe–mineral transformations and their impact on plant growth," Front. Microbiol., vol. 16, no. July, 2025, doi: 10.3389/fmicb.2025.1549022.

[16] R. Shahzad et al., "Biofortification of Cereals and Pulses Using New Breeding Techniques: Current and Future Perspectives," Front. Nutr., vol. 8, no. October, pp. 1–23, 2021, doi: 10.3389/fnut.2021.721728.

[17] S. Kaur, A. Kumari, P. Singh, L. Kaur, N. Sharma, and M. Garg, "Biofortification in Pulses," Adv. Agri-Food Biotechnol., pp. 85–103, 2020, doi: 10.1007/978-981-15-2874-3_4.

[18] S. Lakshmanan et al., "Biofortification in Pulses and Legumes for Alleviating Malnutrition and Enhancing

Nutritional Security: a Review," Appl. Ecol. Environ. Res., vol. 23, no. 1, pp. 889–914, 2025, doi: 10.15666/aeer/2301_889914.

[19] S. Spain, N. Ndlovu, P. C. McKeown, U. Murray, and C. Spillane, "Gender and the adoption of biofortified crop varieties in low and middle-income countries: a review," Front. Sustain. Food Syst., vol. 9, no. January, pp. 1–18, 2026, doi: 10.3389/fsufs.2025.1634171.

[20] S. Venkataraman, D. Zaruk, and K. Hefferon, "Recent developments, challenges and opportunities in genome editing for crop science from a societal perspective," Front. Genome Ed., vol. 7, no. June, pp. 1–14, 2025, doi: 10.3389/fgeed.2025.1568072.

[21] U. Singh, C. S. Praharaj, S. S. Singh, and N. P. Singh, "Biofortification of food crops," Biofortification Food Crop., pp. 1–490, 2016, doi: 10.1007/978-81-322-2716-8.

[22] V. K. Upadhayay et al., "Zinc-solubilizing bacterial consortia: a promising approach for zinc biofortification of crops," Front. Microbiol., vol. 16, 2025, doi: 10.3389/fmicb.2025.1575514.

[23] Y. S. Shivay, U. Singh, R. Prasad, and R. Kaur, "Agronomic interventions for micronutrient biofortification of pulses," Indian J. Agron., vol. 61, pp. 161–172, 2016.

[24] Y. Wang et al., "Revolutionizing Agriculture With CRISPR Technology: Applications, Challenges, and Future Perspectives," Biotechnol. J., vol. 20, no. 9, 2025, doi: 10.1002/biot.70113.

[25] Y. Y. Zhang, R. Stockmann, K. Ng, and S. Ajlouni, "Opportunities for plant-derived enhancers for iron, zinc, and calcium bioavailability: A review," Compr. Rev. Food Sci. Food Saf., vol. 20, no. 1, pp. 652–685, 2021, doi: 10.1111/1541-4337.12669.

LIST OF ABBREVIATIONS

Abbreviation	Full Form
Zn	Zinc
Zn^{2+}	Zinc ion
G × E × M	Genotype × Environment × Management
G × E	Genotype × Environment
QTL	Quantitative Trait Loci
MAS	Marker-Assisted Selection
HTP	High-Throughput Phenotyping
GWAS	Genome-Wide Association Studies
CRISPR/Cas	Clustered Regularly Interspaced Short Palindromic Repeats / CRISPR-associated protein system
PGPR	Plant Growth-Promoting Rhizobacteria
ZSB	Zinc-Solubilizing Bacteria
AMF	Arbuscular Mycorrhizal Fungi
AM	Arbuscular Mycorrhiza
IAA	Indole-3-Acetic Acid
Nano-Zn	Nano Zinc Fertilizer
$ZnSO_4$	Zinc Sulfate
ZnO	Zinc Oxide
GMO	Genetically Modified Organism
ZIP	Zinc-Regulated/Iron-Regulated Transport Protein
HMA	Heavy Metal ATPase
MTP	Metal Tolerance Protein
MT	Metallothionein
PC	Phytochelatin
QTLs	Quantitative Trait Loci (plural)
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
mRNA	Messenger Ribonucleic Acid
MAS	Marker-Assisted Selection
GS	Genomic Selection
HTP	High Throughput Phenotyping
AI	Artificial Intelligence
ML	Machine Learning
G × E × M	Genotype × Environment × Management Interaction
ZnUE	Zinc Use Efficiency
ZnSB	Zinc-Solubilizing Bacteria
P	Phosphorus
pH	Potential of Hydrogen (soil acidity/alkalinity scale)

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