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Metody biofortyfikacji służące do wzbogacania upraw żywnościowych w mikroelementy: przegląd ogólny

Biofortification methods for enriching food crops with micronutrients: a general review

DOI: <http://dx.doi.org/10.12775/KOSMOS.2026.002>

Abstrakt

Biofortyfikacja to forma biotechnologii, która zajmuje się problemem ukrytego głodu, stanowiącego szczególny problem w krajach rozwijających się. Głównym celem tego procesu jest wzbogacenie roślin jadalnych typowych dla danego regionu poprzez zwiększenie stężenia niezbędnych składników odżywczych w postaci przyswajalnej, których niedobór może powodować problemy zdrowotne. Niniejszy artykuł przedstawia kompleksowy przegląd głównych strategii stosowanych w biofortyfikacji roślin w celu zwiększenia stężenia i przyswajalności pierwiastków śladowych w roślinach uprawnych. Szczególną uwagę poświęcono hodowli konwencjonalnej, podejściom agronomicznym, inżynierii genetycznej oraz wykorzystaniu pożytecznych mikroorganizmów, omawiając ich mechanizmy działania, zalety i ograniczenia. Nowatorski charakter niniejszej pracy polega na zintegrowanym porównaniu tych podejść oraz zidentyfikowaniu kluczowych wyzwań i przyszłych kierunków badań w celu skutecznego wdrożenia biofortyfikacji jako strategii zwalczania globalnych niedoborów mikroelementów.

Słowa kluczowe: biofortyfikacja, ukryty głód, hodowla konwencjonalna, praktyki agronomiczne, inżynieria genetyczna, pożyteczne mikroorganizmy

Abstract

Biofortification is a form of biotechnology that addresses the issue of hidden hunger, which is a particular concern in developing countries. The main goal of this process is to enrich the edible plants that are typical of a given region by increasing the bioavailable concentrations of essential nutrients that could cause health problems due to their deficiency. This article presents a comprehensive overview of the main strategies used in plant biofortification to enhance the concentration and bioavailability of trace elements in crop plants. Particular attention is given to conventional breeding, agronomic approaches, genetic engineering, and the use of beneficial microorganisms, with discussion of their mechanisms, advantages, and limitations. The novelty of this work lies in the integrated comparison of these approaches and the identification of key challenges and future research directions for the effective implementation of biofortification as a strategy to combat global micronutrient deficiencies.

Keywords: biofortification, hidden hunger, conventional breeding, agronomic practices, genetic engineering, beneficial microbes

1. INTRODUCTION

Plant biofortification as an idea was conceived around 35 years ago (Fig. 1) and is a targeted agricultural approach aimed at increasing the bioavailable concentrations of essential nutrients in the edible parts of crops. This method enhances the nutritional quality of food products through agronomic practices or genetic modifications (Dhaliwal et al., 2022), promoting broader access to essential nutrients without requiring significant changes in dietary patterns. Unlike conventional food fortification, which involves the post-harvest addition of nutrients during food processing, biofortification enhances the nutrient content directly at the crop level, potentially improving bioavailability of essential nutrients (Liberal et al., 2020; Ofori et al., 2022). Compared to supplementation, which often requires ongoing external inputs and complex logistics, biofortification is a more economically sustainable and cost-effective long-term strategy, particularly in low-income regions (Kurdekar et al., 2023; Mandial et al., 2023; Mushtaq and Nazir, 2021; Ojha et al., 2023; Rai et al., 2024). Enabling farmers to cultivate biofortified crops locally reduces dependence on external aid, strengthens local food systems, and contributes directly to improved nutrition and economic resilience (Mushtaq and Nazir, 2021; Rai et al., 2024). Nevertheless, the successful implementation of biofortification depends not only on the development of nutrient-rich crop varieties, but also on

their acceptance by both farmers and consumers. Despite these challenges, biofortification remains a viable, agriculture-based solution for combatting nutritional deficiencies (Kurdekar et al., 2023; Rai et al., 2024).

Its growing importance is directly connected with the global burden of micronutrient deficiencies, commonly referred to as ‘hidden hunger’. Which affect over two billion people worldwide, particularly in low- and middle-income countries (Lowe, 2021). One of the factors contributing to this problem is the low concentration of essential micronutrients in agricultural soils, which limits their accumulation in edible plant tissues and consequently reduces their dietary intake by humans. Hidden hunger results from diets that are energy-dense but poor in essential nutrients like vitamin A, iron, iodine, selenium, zinc, etc. These deficiencies can lead to serious health consequences, including impaired immune function, developmental delays, reduced productivity, and increased mortality (Huey et al., 2022; Mouli et al., 2024). Vulnerable groups, such as pregnant women, young children and the elderly, are particularly at risk. The highest prevalence is observed in regions such as Latin America, Africa, and South Asia (Skalny et al., 2021). Biofortification mitigates these deficiencies by increasing the concentration of micronutrients in widely consumed and available staple crops such as wheat, maize, potatoes and rice.

In response to these global challenges, numerous biofortification programs and initiatives

have been implemented to combat hunger and mitigate deficiencies of major micronutrients. One of the most popular biofortification programs is HarvestPlus, launched in 2003. It originated from the Biofortification Challenge (BCP) program launched in 2002 by the Consultative Group on International Agricultural Research (CGIAR), a global partnership committed to achieving a food-secure future. The initiative was developed as part of a fast-tracked response to micronutrient deficiencies in low- and middle-income countries. The main goal of HarvestPlus program is to improve nutrition and public health by developing staple food crops rich in essential vitamins and minerals, such as vitamin A, iron, and zinc. By integrating these micronutrients into commonly consumed crops, the program helps address dietary deficiencies in vulnerable populations. To date, it is estimated that approximately 100 million people have benefited from the consumption of biofortified crops made available through the efforts of HarvestPlus (<https://www.harvestplus.org/about/20years>).

Between 2019 and 2022, two organizations, HarvestPlus and the Global Alliance for Improved Nutrition (GAIN) jointly implemented the Commercialisation of Biofortified Crops (CBC) program in six countries across Africa and Asia. The program aimed to increase both the production and consumption of biofortified foods. As part of this effort, nine country-specific crop programs were developed, focusing on the enrichment of local staple foods with essential micronutrients and vitamins, depending on regional nutritional priorities (<https://www.harvestplus.org/what-we-do/projects/cbc-project/>). However, the first biofortification project in Africa was the Africa Biofortified Sorghum Project Consortium, which was launched in 2005. The aim of this consortium was to develop transgenic sorghum varieties that could address multiple nutritional deficiencies by enhancing levels of essential vitamins and increasing the content and bioavailability of amino acids as well as minerals such as iron and zinc (Zhao, 2007).

Despite the growing popularity of biofortification programs, their overall effective-

ness depends on several critical factors, including (I) nutritional impact of the enhanced crop, which varies by micronutrient and crop species, (II) availability of genetic diversity and breeding potential within the target crop, (III) agronomic performance of the biofortified variety under local conditions, (IV) bioavailability and safety of the added nutrients, which must be confirmed, and (V) consumer acceptance (Mouli et al., 2024; Talsma et al., 2017). Furthermore, the long-term success of these initiatives requires strategic planning, efficient resource allocation, and rigorous evaluation of implementation outcomes. To facilitate the effective planning and monitoring of biofortification efforts, the Global Fortification Data Exchange (GFDx) platform was established. GFDx complies and centralized critical data on food fortification programs, serving as a decision-making tool to enhance their design, implementation, and monitoring at national and international levels (<https://www.fortificationdata.org>).

Due to the high demand for a simple solution to 'hidden hunger', biofortification remains a promising technique for delivering various trace elements to areas with depleted diets. The greatest controversy surrounding biofortification is the lack of acceptance of genetic modification of plants in local environments, hence the need to seek or improve other methods. This paper aims to demonstrate the pros and cons of various approaches and propose solutions to existing knowledge gaps with biofortification.

The main goals of this study are:

- I. To provide a critical overview of different approaches to biofortification, including conventional breeding, genetic engineering, agronomic strategies and the use of beneficial microorganisms, with emphasis on their advantages, limitations and practical applicability.
- II. To evaluate the effectiveness, sustainability and implementation challenges of biofortification strategies in the context of global micronutrient deficiencies ('hidden hunger') and to identify future research directions and knowledge gaps.

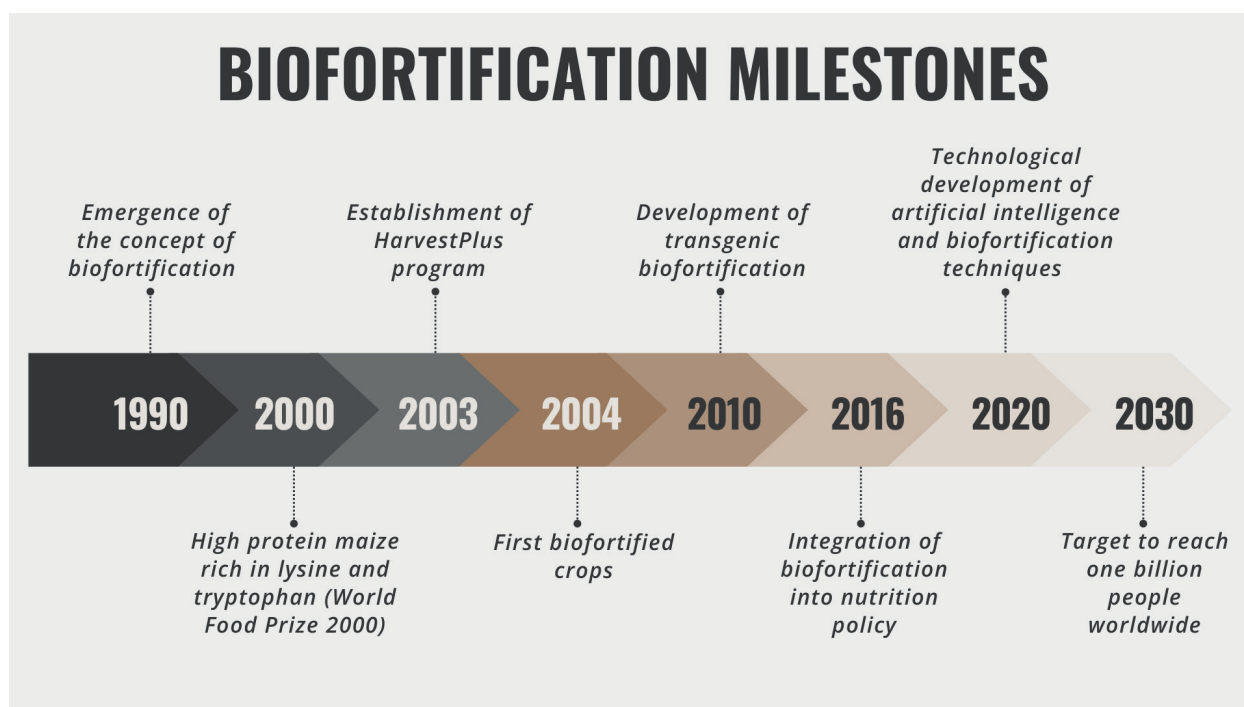


Fig. 1. Biofortification milestones: from concept to current understanding (source: own elaboration)

2. BIOFORTIFICATION STRATEGIES

Biofortification strategies used range from classical approaches, such as conventional breeding and agronomic practices, to advanced genetic engineering methods and the application of beneficial micro-organisms. Table 1 summarises the main assumptions, advantages, limitations and costs associated with these approaches, allowing them to be compared in terms of effectiveness and practical utility.

2.1. Conventional Plant Breeding

The process of conventional plant breeding is based on decision-making: selecting suitable parental individuals, crossbreeding them, and then selecting progeny individuals with desirable traits for further breeding. The process consists of three stages, the first of which is trait mapping, the second is trait introgression and the third is field testing (Kaiser et al., 2020). Trait mapping is the process of identifying and confirming the genetic basis of a trait by finding a related region of DNA. Breeders cross parent

plants with extreme values of a trait and then analyze their offspring, comparing the phenotype (appearance/trait) with DNA markers (genotype). Through successive generations of self-pollination and crossing, they gradually narrow down the location of the gene responsible for the trait. The process requires analyzing thousands of plants over several generations to pinpoint the precise location of the gene in the genome (Kaiser et al., 2020).

Current research indicates that trait mapping, especially using quantitative trait loci (QTL) and fine-mapping methods, can increase the resolution and precision of identifying genetic variants associated with complex traits by accounting for multiple traits, functional annotations, and advanced statistical models, even in the presence of complicated inheritance patterns or genotyping errors (Tong et al., 2021; Wu et al., 2025). A new method for mapping Quantitative Trait Locus (QTL) loci for multiple traits, aims to localize genes affecting quantitative traits and determine their effects on changes in phenotypic traits. With this strategy, based on multi-compartmental mapping, it is possible to simultane-

Table 1. The most important strategies for biofortification, and the advantages and disadvantages of each

Itemisation	Conventional breeding	Agronomic practices	Nano-biotechnology in biofortification	Genetic engineering	Beneficial microbes	References
Basics of methods	Crossing and selecting plants with desirable traits over multiple generations	Crop rotation, irrigation, fertilization, pest and weed management to improve growth conditions	Delivery of micronutrients via nanoparticles (1–100 nm); metallic, oxide, or polymeric formulations and encapsulated nanobiofertilizers	Direct manipulation of plant DNA (e.g., inserting genes for pest resistance, drought tolerance)	Using microorganisms (bacteria, fungi) that promote plant growth or protect against pathogens	Akhtar et al. (2022); Hong et al. (2021); Kaiser et al. (2020); Majumder et al. (2019); Sarmah et al. (2021); Tong et al. (2021)
Advantages	stable production, well adapted varieties; no regulatory restrictions; accepted by consumers	Improves soil fertility, reduces pest pressure, sustainable in long term	Exceptionally high nutrient-use efficiency; high surface area bypasses soil and physiological barriers; dual action: nutrient enrichment and abiotic/biotic stress resilience; lower application doses via eco-friendly green synthesis.	Precise, faster than breeding; allows introducing traits not present in gene pool	Enhances nutrient uptake, increases resilience, eco-friendly alternative to chemicals	Cakmak and Kutman (2018); Das et al. (2022); El-Ramady et al. (2021); Kaiser et al. (2020); Ludwig et al. (2024); Reshma and Meenal (2022); Sab et al. (2020)
Disadvantages	Time-consuming (many years); limited to existing gene pool	Often labor-intensive; effectiveness depends on environment bioavailability	High risk of phytotoxicity and genotoxicity at elevated doses; negative impacts on soil microbiome; risk of human toxicity from over-accumulation	Ethical/regulatory concerns; possible ecological risks; expensive research and development	Variable effectiveness in field; dependent on microbial survival and environment	Cakmak and Kutman (2018); El-Ramady et al. (2021); Kaiser et al. (2020); Ludwig et al. (2024); Reshma and Meenal (2022); Sab et al. (2020)
Costs	Relatively low	Moderate	Variable	High	Moderate	Akhtar et al. (2022); Cakmak and Kutman (2018); Majumder et al. (2019); Reshma and Meenal (2022); Shariatipor et al. (2021); Wu et al. (2025)

ously estimate additive and dominant effects, recombination rates and error rates. The results of simulations and analysis of real data show that the inclusion of genotypic errors improves the accuracy of QTL parameter estimation (Tong et al., 2021). Fine mapping is a method that is used in the field of genetics and aims to accurately identify genetic variants that directly affect the traits of the organism under study. It is a step that follows a genome wide scan (GWAS) to identify regions of DNA associated with a trait or disease. Fine mapping makes it possible to narrow down the regions and determine which specific variants are causal (i.e., actually affect the trait) and which merely coexist due to linkage disequilibrium (LD). This uses functional data, statistical methods, and comparisons between different populations (Wu et al., 2025).

QTL mapping is a widely used method for detecting areas of the genome associated with micronutrient levels. For example, in rice, it has been possible to identify QTLs, such as qZn5.2, which are related to zinc content and can be used to breed varieties enriched in this element (Palanog et al., 2023). Also in wheat, QTLs related to the presence of iron and zinc have been identified, providing valuable information for breeding work on biofortification (Shariatipour et al., 2021).

Meta-QTL analysis is an advanced approach that improves traditional QTL mapping by combining results from different studies to extract stable QTLs with smaller confidence intervals, resulting in greater accuracy in trait identification. In wheat, the technique has been used to detect stable QTLs responsible for grain yield and micronutrient content, supporting work to develop varieties with higher nutritional value (Shariatipour et al., 2021).

Whole genome association analyses (GWAS) are used to detect correlations between genetic markers and specific traits across a broad spectrum of genetic material. In the case of chickpeas, the technique has been used to identify significant correlations between markers and nutritional traits, contributing to the development of biofortified varieties (Sab et al., 2020).

2.2. Agronomic Approaches

Agronomic biofortification involves the application of mineral nutrients to crops to increase the concentration and bioavailability of essential micronutrients in edible plant tissues. It is considered one of the fastest and most cost-effective approaches for addressing micronutrient deficiencies in human populations (Reshma and Meenal, 2022). Nutrients can be supplied through soil or foliar fertilization, although the effectiveness of these methods depends on factors such as crop species, growth stage, environmental conditions, and the chemical form of the applied micronutrient. For example, soil application of ZnSO_4 enhanced grain zinc concentration and yield in wheat and rice. Similar effects were reported for cowpea, chickpea and lentil, where zinc fertilization improved yield, grain Zn concentration and bioavailability while reducing phytate levels. Furthermore, combined soil application of selenium and zinc in soybean increased the accumulation of both micronutrients in seeds, demonstrating the potential of multi-element biofortification strategies (Dhalwal et al., 2022).

Foliar application is often regarded as an effective strategy for increasing the concentration of selected micronutrients, particularly zinc and selenium, due to rapid nutrient uptake and translocation within the plant (Toor et al., 2020). In rice, foliar application of FeSO_4 increased grain iron concentration in different cultivars, while foliar Zn and Fe fertilization enhanced grain Zn concentration and yield traits in maize depending on cultivar-specific absorption efficiency. In wheat, foliar application of Zn, Mn and Fe increased both grain yield and micronutrient concentrations. Furthermore, a foliar mixture containing Zn, I, Se and Fe successfully enhanced grain concentrations of Zn, I and Se in wheat under diverse environmental conditions across six countries. Similarly, foliar application of Zn and Se increased grain Zn and Se concentrations in wheat while reducing Cd uptake under cadmium stress (Prom-U-Thai et al., 2020).

Recent studies have also highlighted the potential of nano-fertilizers to improve nutrient use efficiency and micronutrient delivery in biofortification programs (Hong et al., 2021). For example, studies on ZnO nanoparticles in crop biofortification show consistent improvements in zinc accumulation across different plants. In maize, foliar application of plant-synthesized ZnO-NPs increased grain zinc content by 33% compared to conventional zinc sulfate. In mung beans, biosynthesized ZnO-NPs doubled seed zinc concentration and raised leaf zinc by 63%. Similar effects were observed in wheat, sorghum, and other cereals, where ZnO-NPs improved grain zinc content and overall nutritional quality, particularly in zinc-deficient soils (Semenova et al., 2024). Studies on iron-based nanoparticles in crop biofortification demonstrate significant improvements in both micronutrient content and overall nutritional quality. In broad beans (*Vicia faba* L.), foliar application of Fe₃O₄ nanoparticles increased iron levels by 2.5-fold, zinc by 2-fold, and manganese by 30%, while also enhancing protein and carbohydrate content. Similarly, in wheat, seed treatment with Fe₂O₃ nanoparticles raised iron content by 25% and improved the accumulation of other essential nutrients such as phosphorus and potassium, highlighting their effectiveness in enhancing crop nutritional value (Semenova et al., 2024).

2.3. Nanobiotechnology In Biofortification

Nanobiotechnology provides highly specialized platforms for nutrient delivery using engineered nanoparticles (NPs), which are microscopic particles typically ranging from 1 to 100 nm (Minello et al., 2025; Singh et al., 2025). These nutrient delivery systems feature diverse formulations and can be synthesized from metals such as iron, zinc, copper, and silver, metal oxides like zinc oxide and magnetite (Fe₃O₄), or biocompatible polymers including chitosan, cellulose, sodium alginate, and starch (Akhtar et al., 2022; Minello et al., 2025). A highly innovative extension of these platforms is the “nanobiofertilizer,” which combines engineered nanoparticles with beneficial microbes, such as

plant-growth-promoting rhizobacteria, often by encapsulating the microbial cells within a biodegradable nanomaterial capsule (Akhtar et al., 2022). Unlike conventional bulk fertilizers that dissolve instantly and are easily lost to the environment, these advanced nano-coatings and encapsulations allow for a slow, sustained, and controlled release of nutrients over an extended period (Akhtar et al., 2022; El-Ramady et al., 2021). This target-specific, response-based release ensures that plants receive a steady supply of nutrients synchronized with their precise developmental needs, successfully avoiding sudden concentration spikes and subsequent rapid depletion (Minello et al., 2025).

By utilizing these controlled mechanisms, nanotechnology significantly enhances nutrient-use efficiency (NUE) and overcomes the notorious inefficiencies of conventional bulk fertilizers, which routinely suffer from low solubility, rapid fixation in soils, and high losses through leaching or volatilization (Minello et al., 2025; Singh et al., 2025). Because nanoparticles possess a much higher surface area-to-volume ratio than bulk materials, their solubility, mobility, and reactivity in the soil matrix or on leaf surfaces are vastly improved (Minello et al., 2025; Singh et al., 2025). When applied via foliar spraying, nanofertilizers can achieve exceptional absorption rates of up to approximately 90% (Minello et al., 2025). Due to their nanoscale size, they easily penetrate leaf cuticles, enter through stomata or hydathodes, and translocate through the plant's vascular system directly to target intracellular organs like chloroplasts (Minello et al., 2025). By facilitating such targeted delivery, nanoparticles bypass many soil-related chemical barriers, such as high pH or competing elements that typically render conventional fertilizers unavailable to roots, thereby ensuring that a much larger fraction of the applied nutrient is successfully absorbed and utilized by the plant (Minello et al., 2025).

Consequently, this increased efficiency opens up significant opportunities for nanotechnology-based biofortification, primarily by enabling the highly effective enrichment of edible plant parts such as grains, fruits, and leaves with

vital micronutrients like iron, zinc, and selenium (Minello et al., 2025). Nanoparticle-based approaches have been shown to drastically outperform conventional fertilization in elevating the nutritional profile of staple crops to combat global hidden hunger (Minello et al., 2025). Furthermore, these nanomaterials act as a “nano-umbrella,” providing a dual benefit: they biofortify the plant while simultaneously enhancing its tolerance to severe abiotic stresses, such as drought and salinity, as well as biotic pressures from pathogens (El-Ramady et al., 2021; Singh et al., 2025). At the physiological level, nanoparticles improve plant water retention, boost antioxidant defense systems, and regulate vital phytohormones (El-Ramady et al., 2021; Singh et al., 2025). These systems can also be produced using eco-friendly and cost-effective formulations via “green synthesis” techniques, which utilize plant extracts or microbes to synthesize nanoparticles (Akhtar et al., 2022; El-Ramady et al., 2021). This offers a sustainable, low-toxicity, and economical manufacturing alternative to harsh chemical methods. Because nanofertilizers are so structurally efficient, they require much lower application doses, significantly reducing input costs for farmers while lowering the overall chemical burden on the agricultural environment (El-Ramady et al., 2021).

2.4. Genetic Engineering

Genetic engineering methods used in biofortification are advanced biotechnological approaches aimed at increasing the nutritional value of crops through targeted modification of plant genomes. These techniques enable the precise enrichment of staple foods with essential micronutrients such as iron, zinc, iodine, and selenium, particularly in situations where conventional breeding is limited by insufficient genetic variability. Genetic engineering therefore provides a rapid and directed strategy for developing nutrient-rich crops and addressing global micronutrient deficiencies. The main molecular strategies include enhancing mineral uptake from soil through overexpression of metal transporter genes (e.g., *IRT1* or *OsVIT*) (Balyan

et al., 2020; Majumder et al., 2019), improving internal transport via chelators such as nicotianamine synthase (NAS), increasing nutrient storage in edible tissues through proteins like ferritin (Majumder et al., 2019; Masuda et al., 2013). Additionally, metabolic engineering can enhance vitamin biosynthesis, for example by modifying pathways leading to β -carotene production through *psy* and *crtI* genes (Garg et al., 2018; Majumder et al., 2019). A prime example of this approach is Golden Rice, where engineering the β -carotene biosynthetic pathway resolved the lack of vitamin A in the endosperm of traditional rice. Under initiatives like HarvestPlus, similar metabolic engineering has led to the development of Quality Protein Maize (QPM) and other vitamin-biofortified crops aimed at mitigating malnutrition in developing nations (Debnath et al., 2026). Classical genetic engineering approaches include transgenesis, cisgenesis, and metabolic engineering, which enable the insertion, modification, or transfer of genes to achieve desired nutritional traits (Shi et al., 2025; Simkin, 2019).

While advanced biotechnology offers precise solutions, conventional and molecular breeding programs (such as the Indian AICRIP) have also successfully yielded biofortified varieties, including high-protein and high-zinc rice (e.g., CR Dhan 310, DRR Dhan 45) and zinc-enriched wheat (e.g., WB 02, HPBW 01, BHU 1), designed to overcome the “genetic dilution” of micronutrients caused by the modern, high-yielding crops of the Green Revolution. However, to maximize the nutritional impact of both conventional and engineered crops, reducing anti-nutritional factors remains critical (Debnath et al., 2026).

Next-generation technologies (NGTs) represent an extension of these genetic engineering approaches, incorporating highly precise genome editing tools such as CRISPR/Cas9, TALENs, and ZFNs (Banerjee et al., 2023; Ibrahim et al., 2021). Rather than only increasing mineral accumulation directly, many successful applications focus on improving micronutrient bioavailability by reducing anti-nutritional compounds, especially phytic acid, which strongly

chelates iron and zinc in cereal grains (Banerjee et al., 2023; Ibrahim et al., 2021). For example, zinc finger nucleases (ZFNs) have been used in maize to silence the ZmIPK1 gene involved in phytate biosynthesis, resulting in reduced phytic acid content and improved mineral bioavailability (Ibrahim et al., 2021). Similarly, TALEN- and CRISPR/Cas9-mediated editing of genes such as ZmIPK1A and ZmMRP4 has been shown to significantly lower phytate levels in maize, while also enabling additional modifications of quality traits in rice (Ibrahim et al., 2021). Beyond reducing anti-nutritional factors, genome editing tools have also been used to directly enhance micronutrient uptake and yield. For example, alteration of the OsNAS2 promoter in rice using CRISPR-based approaches has been shown to increase zinc uptake and improve yield (Ludwig et al., 2024). Importantly, NGTs offer a major advantage over traditional transgenic approaches, as they can generate targeted mutations without introducing stable foreign DNA, making edited plants more similar to naturally occurring variants. This increases their potential acceptance and positions NGTs as a promising GMO-free strategy for future biofortification and sustainable crop improvement (Banerjee et al., 2023; Ibrahim et al., 2021).

Despite the technological prowess of these tools, the real-world deployment of biofortified crops faces significant social and regulatory hurdles. Genetically modified organisms (GMOs) continue to encounter deep public distrust and low acceptance globally. Golden Rice serves as a classic historical example, where public apprehension, disinformation campaigns, and anti-GMO vandalism delayed its commercialization for nearly two decades (Debnath et al., 2026; Zhou et al., 2023). Furthermore, the development of transgenic crops involves rigorous, non-harmonized international regulatory frameworks that incur immense economic costs and timelines. From a biosafety perspective, regulatory bodies strictly demand the absence of bacterial marker genes, such as antibiotic resistance genes, in the final food product. Environmental and physiological concerns also persist; for instance, manipulating plant iron homeostasis can

trigger cellular oxidative stress and potentially alter natural disease resistance. In this landscape, transgene-free crops edited via CRISPR benefit from a more favorable regulatory classification in certain jurisdictions like the United States, although they remain strictly regulated as traditional GMOs within the European Union. Navigating these socio-regulatory challenges is essential for utilizing NGTs as a pillar of sustainable global nutrition (Debnath et al., 2026; Nath et al., 2026).

2.5. The Use Of Beneficial Microbes

Biofortification with beneficial micro-organisms is based on the use of plant growth-promoting bacteria (PGPB) and mycorrhizal fungi. Among other things, these micro-organisms can mobilize nutrients from soil forms that are difficult to access, as well as stimulate physiological mechanisms in plants that favour their efficient uptake (Jalal et al., 2025; Wahab et al., 2023). This method is an environmentally friendly alternative to chemical mineral fertilizers, reducing costs and negative environmental impacts. However, its limitation is the variability of its effectiveness under field conditions due to differences in the composition of the soil microbiome and environmental factors (Jalal et al., 2025).

The main mechanisms used by plant growth-promoting microorganisms (PGPMs) include several key processes that enhance plant nutrition and development. One mechanism is the inoculation of bacteria that solubilize microelements, which release micronutrients from forms that are otherwise difficult for plants to access; including, among others *Bacillus*, *Pseudomonas*, *Azospirillum*, *Rhizobium*, and *Enterobacter* (Jalal et al., 2025). For example, inoculation of wheat with a bacterial strain closely related to *Bacillus pichinotyi* (YAM2) significantly improved plant biomass and developmental traits such as shoot and spike length, both in the presence and absence of selenate fertilization. Moreover, bacterial inoculation markedly increased selenium accumulation in wheat kernels (by 167%) and stems (by 252%), as well as iron content in kernels (by 70%) and stems (by

147%) compared to non-inoculated plants (Yasin et al., 2015).

Another important mechanism is the production of siderophores, compounds that bind and mobilize iron for plant uptake, as seen in *Pseudomonas fluorescens* and *Azotobacter vinelandii* (Soares, 2022). In *P. fluorescens*, pyoverdines act as the major iron-scavenging system, while in *A. vinelandii* multiple siderophore classes support the high iron demand associated with nitrogen fixation. Mobilized iron is subsequently made available to plants either through reduction of Fe(III)–siderophore complexes at the root surface or via ligand exchange with plant-derived chelators, facilitating iron uptake and improving overall plant nutrition (Timofeeva et al., 2022).

Arbuscular Mycorrhizal Fungi (AMF) form symbiotic relationships with plant roots, increasing the uptake of nutrients such as phosphorus and zinc, as well as improving water absorption; notable species include *Glomus intraradices* and *Rhizophagus irregularis* (Wahab et al., 2023). For example, inoculation with *R. irregularis* has been shown to increase nutrient accumulation and root biomass while helping plants tolerate heavy metal stress, including cadmium and zinc exposure. Similarly, *G. intraradices* enhances phosphorus content and root dry weight in sunflower even under contamination with heavy metals such as zinc, copper, and lead (Wahab et al., 2023).

3. CHALLENGES AND CONSTRAINTS OF DIFFERENT BIOFORTIFICATION APPROACHES

3.1. Technical And Agronomic Challenges

Limitations of conventional breeding

The efficiency of classical selection and backcrossing is determined by the natural genetic variation within the gene pool of a given species or its wild relatives. In many cases, this variation is insufficient to achieve therapeutic levels of micronutrients (as exemplified by the natural absence of provitamin A in rice endosperm or inadequate iron accumulation) (Dhaliwal et al., 2022; Majumder et al., 2019; Qureshi et al., 2026).

A significant barrier is also posed by negative genetic correlations resulting from pleiotropy; selection targeted at increasing micronutrient concentration often correlates with a polygenic decline in yield potential or a reduction in quality parameters, such as storage protein content. Furthermore, this process is characterized by low time efficiency, requiring multi-year crossings to eliminate negative linked traits inherited from wild forms (linkage drag) (Dhaliwal et al., 2022; Majumder et al., 2019).

Barriers to agronomic biofortification

The efficiency of soil application of micronutrient fertilizers depends on the physicochemical properties of the soil, particularly its pH (Dhaliwal et al., 2022; Reshma and Meenal, 2022). Under alkaline conditions, soil properties induce ion immobilization (e.g., of iron), converting them into forms unavailable to the root system (Dhaliwal et al., 2022; Qureshi et al., 2026; Reshma and Meenal, 2022). Conversely, foliar application (spraying) poses a risk of phytotoxicity; using salt solutions at higher concentrations can induce leaf blade necrosis, secondary to reducing photosynthetic efficiency and crop yield (Dhaliwal et al., 2022; Qureshi et al., 2026; Reshma and Meenal, 2022). Ion antagonisms at the cellular transporter level also present a serious challenge. It has been shown that exogenous iron application can competitively inhibit zinc translocation to the grain (and vice versa), while high soil phosphorus availability drastically limits zinc uptake (Dhaliwal et al., 2022).

Unintended consequences in transgenic and edited crops

Overexpression of genes encoding metal transporters (e.g., genes from the *VIT* – *Vacuolar Iron Transporter* family) to increase iron and zinc accumulation carries the risk of simultaneous uptake of phytotoxic and carcinogenic heavy metals, such as cadmium, especially when crops are grown on anthropogenically contaminated soils (Majumder et al., 2019). Furthermore, excessive intracellular concentration of labile iron induc-

es oxidative stress through the Fenton reaction. The reactive oxygen species (ROS) generated in this process lead to structural damage to cells, which limits phytomass and lowers plant resistance to biotic stresses. Basic knowledge regarding the mechanisms of mineral homeostasis and element distribution to edible parts also remains limited (Balyan et al., 2020; Gupta et al., 2024).

Limitations of nanobiotechnology

The effectiveness of nanoparticles is highly dose-dependent. While low concentrations stimulate growth, elevated levels trigger severe phytotoxicity and genotoxicity, leading to chromosomal abnormalities, oxidative damage, and abnormal seedling morphology. Additionally, scaling up production to an industrial level faces critical challenges regarding material reproducibility, particle aggregation, and high manufacturing costs (Akhtar et al., 2022; El-Ramady et al., 2021).

3.2. Environmental Challenges

Conventional agronomic fertilization is characterized by a low nutrient recovery rate (e.g., zinc recovery efficiency from soil ranges from just 1.2% to 5.3%). This inefficiency leads to the waste of agrochemicals and their uncontrolled accumulation in the edaphone, destabilizing the ecological and microbiological balance of soils. The alternative use of organic matrices (composts, sewage sludge, manure) as micronutrient sources carries the risk of introducing pathogens, xenobiotics, and heavy metals into agroecosystems (Dhaliwal et al., 2022; Qureshi et al., 2026; Reshma and Meenal, 2022).

The implementation of innovative nanofertilizers, despite their high bioavailability, raises justified ecotoxicological concerns (Dhaliwal et al., 2022). Elevated concentrations of nanomaterials exhibit phytotoxic effects, and the long-term fate of nanoparticles in the environment, their tendency for bioaccumulation in the trophic chain, and their cytotoxic impact on non-target soil microorganisms require rigorous risk assessment prior to widespread com-

mmercialization (Dhaliwal et al., 2022; Reshma and Meenal, 2022).

3.3. Socio-Economic Issues

The development, validation, and implementation of biofortified transgenic lines require sophisticated laboratory facilities and analytical infrastructure (such as ICP-MS apparatus for elemental profiling), which are often lacking in developing countries with the highest rates of malnutrition (Majumder et al., 2019).

For smallholder farmers in regions affected by hidden hunger, cyclical agronomic biofortification (requiring the purchase and application of fertilizers in every growing cycle) represents an excessive financial and logistical burden. The use of synthetic mineral chelates generates particularly high costs (Dhaliwal et al., 2022). The deficit of systemic state support instruments (such as subsidies for certified seed material and dedicated fertilizers), combined with the dysfunction of local seed distribution systems and storage infrastructure, significantly limits the adoption of these technologies by farmers (Bouis et al., 2024).

The widespread adoption of nano-biofortification is constrained by high initial manufacturing costs and technical bottlenecks in large-scale production, which create significant economic uncertainty for commercial agricultural deployment (Akhtar et al., 2022; El-Ramady et al., 2021).

3.4. Regulatory Challenges

The main implementation barrier for biotechnology-based biofortification lies in restrictive and inconsistent legal frameworks. The classic paradigm of these limitations is “Golden Rice” with provitamin A biosynthesis; over two decades elapsed from its laboratory development to the stages of commercialization in the Philippines or Bangladesh, resulting from complex legislative procedures, political barriers, and destructive activities by anti-GMO movements (Debnath et al., 2026; Majumder et al., 2019).

Regulatory requirements force researchers to employ complex genetic engineering strate-

gies aimed at removing bacterial marker genes (such as antibiotic resistance), which constitute a key point of contention in variety authorization processes (Majumder et al., 2019). Furthermore, the legal status of modern genome editing tools (NBTs - *New Breeding Techniques*), such as the CRISPR/Cas9 system, remains inconsistent globally (Shohael et al., 2025). While regulatory agencies in the US have exempted many crops subjected to precise editing (SDN-1/SDN-2) from the rigors of GMO legislation, the Court of Justice of the European Union has equated the status of edited plants with classic transgenic organisms, drastically limiting their implementation in European agriculture (Majumder et al., 2019; Nath et al., 2026).

Nanotechnology faces a severe lack of comprehensive regulatory frameworks and long-term risk assessments. The absence of standardized international guidelines regarding the ultimate fate of nanomaterials in the food chain creates critical bottlenecks for approval and commercialization (Akhtar et al., 2022; Minello et al., 2025).

3.5. Consumer Acceptance And Dietary Issues

The lack of widespread public acceptance of genetically modified food, stimulated by misinformation campaigns, induces a high market risk of consumers rejecting biofortified products (Karaduman et al., 2026; Majumder et al., 2019; Ofori et al., 2022). These concerns are compounded by the specificity of regional sensory and culinary preferences. For instance, polished (white) rice is widely preferred in Asian countries due to its desirable organoleptic properties and shorter thermal processing time (Karaduman et al., 2026; Majumder et al., 2019; Qureshi et al., 2026). However, the mechanical polishing process removes the micronutrient-rich aleurone layer and the embryo, depriving the grains of most iron, zinc, and vitamins. Designing biofortification strategies therefore requires precise targeting of mineral transport to the endosperm, ensuring that processing does not undo the enrichment effect (Karaduman et al., 2026; Majumder et al., 2019).

The ultimate efficacy of the method is limited by the low bioavailability of micronutrients caused by the presence of absorption inhibitors. Cereal grains and legume seeds are characterized by high concentrations of phytic acid, which acts as a strong chelating agent that binds divalent ions (Fe^{2+} , Zn^{2+}) into insoluble complexes (phytins), preventing their absorption in the human gastrointestinal tract (Balyan et al., 2020; Dhaliwal et al., 2022; Qureshi et al., 2026). Consequently, comprehensive biofortification requires simultaneous modifications targeted at silencing phytic acid biosynthesis pathways or overexpressing thermostable phytases to accelerate their enzymatic degradation during food processing (Dhaliwal et al., 2022; Majumder et al., 2019).

Because nanoparticles highly effectively translocate elements into edible tissues, there is a persistent risk of exceeding safe dietary limits. For example, excessive application can push nutrient levels past tolerable upper intake limits, potentially causing over-accumulation toxicity, such as selenosis, in human consumers and livestock (El-Ramady et al., 2021).

4. CONCLUSIONS AND FUTURE PERSPECTIVES

Despite progress, there are still significant gaps in knowledge limiting global biofortification. In the field of plant biology and genetics, knowledge about the mechanisms of mineral homeostasis, nutrient transport in plants, and their distribution to edible parts remains limited, particularly for elements such as iron, zinc, and selenium (Balyan et al., 2020; Gupta et al., 2024). Modern programs must address the crosstalk between iron levels and plant immunity (ferroptosis), which necessitates disease screening. Furthermore, the complex interaction between nutrients, anti-nutrients (such as phytates), food processing, and preparation affects the final bioavailability of micronutrients in the diet. To bypass genetic trade-offs, mapping meta-quantitative trait loci (MQTLs) for marker-assisted selection is essential (Shariatipour et al., 2021).

Future research should focus on developing integrated biofortification strategies that combine advances in plant breeding, modern genome

editing technologies, and sustainable agronomic practices. While the development of technologies such as CRISPR creates new opportunities for the precise modification of traits related to micronutrient uptake and accumulation, precise edits (like the *OsNAS2* promoter) can enhance zinc without transgenes. However, their implementation requires further refinement of regulatory, environmental, and ethical frameworks (Ludwig et al., 2024; Shohael et al., 2025).

In the agronomic sector, particularly promising avenues include the use of plant growth-promoting microorganisms and the application of nanotechnology in micronutrient fertilization, although these approaches still require extensive field testing (Dhaliwal et al., 2022). Nanofertilizers (ENMs) drastically improve nutrient recovery but require environmental risk assessments. Simultaneously, green technologies using siderophore-producing bacteria or arbuscular mycorrhizal fungi maximize soil nutrient availability, while advanced multi-element spectral analysis (ICP-OES/XRF) optimized by genetic algorithms will enhance monitoring (Kuai et al., 2023).

Significant challenges also exist in the social, economic, and political dimensions. Severe metabolic disorders will rely on clinical advancements, such as lipophilic chelators designed via artificial intelligence (AI) (Kontoghiorghe, 2024). Furthermore, there is still a lack of comprehensive analyses of the long-term cost-effectiveness of biofortification compared to other nutritional interventions, as well as research on effective seed distribution systems and the large-scale implementation of biofortified varieties (Bouis et al., 2024). Overcoming this requires the integration of biofortification programs into national food and agricultural policy strategies and ensuring stable funding and institutional support for such initiatives (Kontoghiorghe, 2024).

In the long term, biofortification may become an important element of global strategies to improve food and nutrition security. However, its effectiveness should be assessed not only on the basis of the micronutrient content of plants, but also taking into account their bioavailability, food safety, and social acceptance. Implementing more effective communication and ed-

ucation strategies is crucial, and the integration of agricultural, nutritional, and socio-economic research will be crucial to fully exploit the potential of biofortification in addressing global micronutrient deficiencies.

STATEMENTS

I declare that I am the sole author of this manuscript, that I approve the final version, and that I have no conflict of interest in relation to the preparation and publication of this article.

I would like to express my sincere thanks to Dr. Karina Krzciuk and Professor Sabina Dołęgowska, PhD, of the Jan Kochanowski University, for their valuable substantive comments and for reviewing this manuscript, which have contributed to its final form.

This manuscript did not receive any external funding.

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