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Biofortification of vegetables: Strategies, challenges and future prospects

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Abstract
Micronutrient malnutrition, often termed 'hidden hunger' remains a critical global health issue, particularly in developing regions where plant-based diets dominate. Biofortification of vegetables offers a promising, sustainable and cost-effective strategy to combat this challenge by enhancing the concentration and bioavailability of essential nutrients such as iron (Fe), zinc (Zn) and iodine (I) in widely consumed crops. Vegetables, due to their rich nutrient profile, short growth cycles and wide consumption are ideal candidates for biofortification through agronomic methods, conventional breeding and genetic engineering. Biofortified vegetables contribute not only to improved public health but also to crop quality and resilience. However, successful implementation requires addressing agronomic, environmental and socio-economic challenges, including nutrient bioavailability, consumer acceptance, and policy integration. With growing global food and nutrition demands, vegetable biofortification emerges as a vital complementary strategy toward achieving food security and combating micronutrient deficiencies.

Keywords: Vegetables, antioxidants, biofortification, micronutrient deficiency, genetic engineering

Introduction

Micronutrient malnutrition affects over half the global population and is a major challenge. By 2050, with a population exceeding 9 billion, sustainable food production is crucial. Plant breeders have mainly worked to increase crop yields in recent decades to feed poor populations. However, this focus ignored the nutritional quality of vegetables, leading to many varieties with low nutrition. Now, there is greater awareness that nutritious food is key to fighting malnutrition. Malnutrition is a serious global problem, especially in poorer countries, caused by diets that lack essential nutrients (Yadava *et al.*, 2018) [36]. For good health, people need various minerals from food, especially vegetables. Lack of certain minerals can harm health. Since plant-based diets provide these minerals, it is important to eat balanced and nutritious foods regularly.

One effective strategy to alleviate this issue is biofortification, which involves breeding essential nutrients directly into food crops. This approach offers a cost-efficient, sustainable, and long-lasting solution to deliver higher micronutrient levels, especially benefiting rural populations in developing countries who often have limited access to diverse and nutrient-rich diets (Kumari *et al.*, 2022) [18]. This process of increasing the nutrient content of crops through breeding or genetic engineering is known as biofortification (Neste *et al.*, 2006) [22].

Biofortification improves the nutritional quality of staple crops by increasing nutrient content or availability through farming techniques, breeding, or biotechnology. While biofortified crops may have lower nutrient levels than supplements or industrially fortified foods, they help boost micronutrient intake across populations at all life stages. (Bouis *et al.*, 2010) [6].

Biofortification is a form of food fortification that involves enhancing the concentration and bioavailability of essential nutrients in food crops during their growth and development. Biofortification of vegetable crops is the process of increasing essential micronutrients and vitamins, including trace elements, in vegetable varieties to improve the nutritional quality of the food supply and promote public health. This natural enhancement offers a sustainable and efficient means to provide nutrient-rich foods, particularly in remote rural regions where access to commercially fortified products is scarce.

As a result, biofortification is a vital strategy to combat micronutrient deficiencies and enhance overall nutrition (Singh *et al.*, 2021) [33].

Nutritional Significance of Vegetables

Vegetables are a rich source of essential nutrients-vitamins, minerals, fiber, and bioactive compounds that support overall health, prevent chronic diseases, and maintain physiological functions. Unlike fruit, which often contains higher sugars, vegetables contribute micronutrients and fiber with low calorie density, making them essential for managing weight and disease risk (Table 1.)

Table 1: Sources of nutrients from vegetables (Gomati *et al.*, 2017) [14]

Nutrients	Vegetables
Calcium	Hyacinth bean, amaranthus, and palak
Carbohydrate	Sweet potato, potato, and cassava
Iodine	Tomato, sweet pepper, carrot, garlic, and okra
Iron	Amaranthus, palak, spinach, lettuce, and bitter gourd
Phosphorous	Pea, lima bean, taro, and drumstick leaves
Protein	Pea, lima bean, French bean, and cowpea
Sodium	Celery, green onion, Chinese cabbage, and radish
Vitamin A	Carrot, spinach, and pumpkin
Vitamin B	Palak, amaranthus, bitter and pointed gourd
Vitamin B1	Tomato, chilli, garlic, leek, and pea
Vitamin C	Chilli, sweet pepper, cabbage, and drumstick

Vegetables-A rich source of vitamins

Vegetables play a vital role in human health, as they are rich sources of essential nutrients, vitamins, and phytochemicals that can help reduce the risk of chronic diseases. Poor nutrition, combined with modern lifestyle habits and unhealthy diets, contributes to the development of serious health conditions such as diabetes, obesity, certain types of cancer, stroke, inflammation, and other cardiovascular diseases (Comert and Mogol, 2020) [9]. Vitamins are essential for human health, growth, and development. As previously mentioned, the human body cannot synthesize most vitamins, so they must be obtained through external sources such as plants and animals.

Vitamin A (Carotenoids)

Many vegetables, especially those that are green, orange, or yellow contain carotenoids like beta-carotene, which are converted into vitamin A in the body. Vitamin A is critical for vision (especially in low light), epithelial integrity, growth, and immune defense. Deficiency can lead to xerophthalmia and increased susceptibility to infections. Carrots, sweet potatoes, spinach, and bell peppers are particularly rich in carotenoids.

Vitamin C

Ascorbic acid is abundant in vegetables like bell peppers, broccoli, Brussels sprouts, kale, cabbage, and tomato. Vitamin C supports collagen synthesis (crucial for skin, bone, and blood vessels), enhances iron absorption, acts as an antioxidant, and slows aging processes. Broccoli, yellow/red sweet peppers, kale etc. are good sources of vitamin C.

Vitamin K

Vitamin K, especially phylloquinone (K1), is vital for blood clotting and bone health. Dark leafy greens such as kale, spinach, swiss chard, and collard greens are excellent sources of vitamin K, essential for blood clotting and bone metabolism. Biofortification enhances vitamin K content through breeding, fertilization, or genetic approaches to combat deficiencies. For instance, optimized nitrogen fertilization in spinach increases K levels, improving its nutritional value and supporting public health. Spinach biofortified via nitrogen management showed higher vitamin K1 content (Booth *et al.*, 2003) [5].

Vitamin B Complex

Vitamin B complex includes essential water-soluble vitamins like B1 (thiamine), B2 (riboflavin), B6, B9 (folate), and B12, vital for energy metabolism, nerve function, and red blood cell formation. Many vegetables are low in B vitamins, especially B12. Biofortification through breeding, genetic engineering, or microbial symbiosis enhances B vitamin levels in Vegetables crops. Vitamin B6 is present in moderate amounts across vegetables (e.g., spinach, cabbage, carrots, potatoes). Lettuce biofortified with folate (B9) via metabolic engineering showed increased folate levels (Diaz de la Garza *et al.*, 2007) [11].

Vegetables-A rich source of minerals

Vegetables are a rich source of essential minerals vital for human health. They provide key minerals such as potassium, magnesium, calcium, iron, and zinc, which support various bodily functions including muscle contraction, nerve transmission, bone health, and immune function. Vegetables can be enhanced to contain higher levels of essential minerals such as iron, zinc, calcium, and selenium (Table 2). Regular consumption of a variety of vegetables helps maintain mineral balance and supports overall health, making them a crucial part of a balanced diet.

Table 2: Mineral availability in various vegetable crops (Gomati *et al.*, 2017) [14]

Vegetable Crops	Minerals
Brassica spp.	Selenium
Carrot	Calcium
Cassava	Zinc, Selenium and Copper
Cucumber	Potassium
Amaranth	Iron
Onion, Broccoli	Selenium
Pumpkin	Zinc and Phosphorus
Radish	Selenium
Tomato	Zinc and Iodine

Target Minerals and Their Impacts

Iron (Fe)

Iron (Fe) is an essential micronutrient required by humans, animals, and plants. As a redox-active metal, it plays a vital role in catalyzing oxidation-reduction reactions and in regulating cell growth and differentiation. Iron is a key component of numerous proteins and enzymes, where it functions as a cofactor essential for various physiological processes. Iron in vegetables plays a crucial role in human nutrition by supporting oxygen transport, energy metabolism, and immune function. Although vegetables provide non-heme iron, its bioavailability is often limited by compounds like phytates and oxalates. Cooking, fermentation, and combining vegetables with vitamin C rich foods can enhance iron absorption. Leafy greens such as spinach, cabbage, and legumes are key sources. Promoting iron-rich and biofortified vegetables is vital for addressing dietary iron deficiency.

Zinc (Zn)

Zinc (Zn) plays a vital role in human health, contributing to the structural integrity and catalytic activity of numerous enzymes. It is essential for the synthesis of nucleic acids and proteins, and it supports key biological processes such as cell differentiation, glucose metabolism, and insulin secretion (Roohani *et al.*, 2013). Vegetables contribute to dietary zinc intake, though the bioavailability is lower due to inhibitors like phytates. Leafy vegetables such as spinach, cabbage, and broccoli contain ~2.8-4.5 mg/kg zinc, while legumes can reach 3-6 mg/kg. Tomatoes and bell peppers contain less (~0.5-1.5 mg/kg). Enhancing zinc absorption from vegetables is possible through soaking, sprouting, or combining with acidic foods. Vegetable zinc content varies widely but remains nutritionally significant. Zinc biofortification, particularly through the application of zinc sulphate ($ZnSO_4$), has shown promising results in enhancing the mineral content of vegetable crops (Buturi *et al.*, 2021) [7]

Selenium (Se)

Selenium (Se) is a vital micronutrient for human health, playing key roles in antioxidant defense and immune function. However, its content in vegetables is often low due to Se-deficient soils. Biofortification enhancing Se levels in crops through agronomic practices (e.g., Se-enriched fertilizers), genetic selection, or microbial interaction can effectively improve Se intake in human diets. In vegetables, Se also supports plant growth, enhances stress resistance, and improves post-harvest quality. Se enriched vegetables such as broccoli, garlic, and spinach have shown increased health benefits. Sustainable Se biofortification strategies are essential to combat global Se deficiency and support public health (White and Broadley, 2009) [34].

Iodine (I)

Iodine is an essential micronutrient that plays a vital role in human metabolism. A deficiency in iodine can result in serious thyroid disorders and impair the proper synthesis of thyroid hormones (Dai *et al.*, 2004) [4]. These hormones are crucial for regulating various metabolic processes. The recommended daily allowance (RDA) of iodine ranges from 40 to 200 μg per day, depending on age, sex, and physiological conditions. Consuming iodine-rich vegetables such as spinach, potatoes, and seaweed can help meet dietary requirements.

Types of Biofortication (Strategies for biofortification of plant-based foods)

Biofortification can be achieved through three main strategies: agronomic biofortification, biofortification through conventional breeding and biofortification through genetic engineering.

Agronomic biofortification

Agronomic biofortification involves the application of micronutrient enriched fertilizers to enhance the nutrient density of crops. It is an efficient and rapid approach to improve crop nutritional quality, and the consumption of such biofortified crops contributes to better human micronutrient intake and overall nutritional status (Cakmak and Kutman, 2018) [28]. Agronomic biofortification primarily depends on fertilizer application methods, as well as the solubilization and translocation of mineral elements from the soil to the edible parts of the. The micronutrients enhance nutritional quality and macronutrients such as nitrogen (N), phosphorus (P), and potassium (K) play a crucial role in supporting plant (Bhardwaj *et al.*, 2022) [3].

The application of micronutrients through fertilizers can enhance soil micronutrient availability and help correct deficiencies in both plants and humans. However, in many cases, the applied micronutrients become quickly immobilized in the soil, limiting their uptake and translocation to the edible parts of the plants. One major benefit of agronomic biofortification is that it is not limited to a specific crop. Micronutrient fertilizers and their application methods can be easily adjusted and used across different crops. On the other hand, genetic and transgenic biofortification are crop-specific processes, which makes expanding biofortified traits to multiple crops slow, costly, and labor-intensive

Targeted vegetables crops for biofortification through agronomic practices

Field pea is well known for its high protein content and can be biofortified with zinc through foliar application, either alone or in combination with soil-based zinc treatments. (Poblaciones *et al.*, 2016) [26]. The uptake of Copper (Cu), Manganese (Mn), and Zinc (Zn) in common beans was enhanced through the application of both organic and synthetic fertilizers (Ram *et al.*, 2016) [28]. Chickpea biofortification through mycorrhizal association has been effective in addressing iron and zinc deficiencies (Pellegrino and Bedini, 2014) [24].

Biofortification through conventional breeding

Plant breeding (conventional breeding) involves developing genetically improved or new crop varieties with enhanced levels of essential micronutrients to address nutritional deficiencies. (Sheoran *et al.*, 2022; Shahzad *et al.*, 2021) [32, 30]. Biofortification through plant breeding aims to improve the concentration and bio accessibility of minerals in crops by utilizing genetic differences among varieties of the same species. Initially, plant breeding focused on increasing yield and enhancing agronomic traits. However, recent breeding techniques are now geared. Conventional breeding is the most common and widely accepted method of plant breeding for biofortification. It enhances the nutritional quality of crops without compromising other important agronomic traits. Biofortification through conventional breeding involves crossing crop varieties that possess high nutrient density with those that have desirable agronomic traits, to produce new varieties with improved nutritional and agronomic characteristics (Garg *et al.*, 2018) [13]. This process requires identifying biodiverse crop varieties, assessing the traits and levels of target nutrients, and evaluating the effects of growing conditions on the stability of these traits (Shelenga *et al.*, 2021) [31].

Mutation breeding differs from conventional breeding in that it generates genetic variation by inducing mutations through chemical agents or physical methods such as irradiation. In contrast, conventional breeding relies on naturally existing genetic diversity within or between plant populations. Mutation breeding introduces novel genetic variations by directly altering the plant's DNA, thereby expanding the pool of traits available for selection.

Biofortification through molecular breeding involves identifying the genomic location of genes associated with improved nutritional quality, along with molecular markers closely linked to these genes. These markers enable the precise selection and incorporation of desirable traits into crop varieties through conventional breeding methods, thereby accelerating the development of nutritionally enhanced cultivars (Jha and Warkentin, 2020) [17]. Molecular breeding can be used to determine if a desirable trait is present or absent in a specific crop during developmental stages. Hence, it is more rapid compared to other forms of plant breeding.

Conventional plant breeding approaches can benefit not only large populations but also people living in remote rural areas with limited access to commercially marketed fortified foods (Bouis *et al.*, 2010) [6]. This approach requires a one-time investment in plant breeding, after which the crops can be grown and multiplied by farmers over the years at virtually zero marginal cost. Recurrent costs remain low, and germplasm can be shared internationally without negatively affecting productivity or health. As a result, biofortification enjoys broad public acceptance (Nestel *et al.*, 2006; Winkler, 2011) [22, 35].

Targeted vegetables crops for biofortification through conventional plant breeding:

Sweet Potato: The primary goal of biofortification programs in sweet potatoes is to replace white-fleshed varieties, which are low in provitamin A, with orange-fleshed varieties rich in beta-carotene. Harvest plus has set a target beta-carotene concentration of 32 µg/g in sweet potato roots, although some varieties already contain up to 100 µg/g (Nestel *et al.*, 2006) [22].

Beans: Beans are often termed the "poor man's meat" due to their affordability and rich content of protein, iron, zinc, and essential vitamins. Mineral biofortification of common beans is crucial to combat micronutrient deficiencies in low-income populations. This process involves several stages, including germplasm screening, studies on trait inheritance and plant physiology, assessment of mineral bioavailability, and the development of improved biofortified varieties suitable for widespread cultivation and consumption (Blair, 2013) [4].

Biofortification through genetic engineering

Genetic engineering is the preferred approach when there is limited or no genetic diversity for essential nutrients within crop species (Perez-Massot *et al.*, 2013) [25]. This method allows for the transfer and overexpression of desirable heritable traits from unrelated plant species or even non-plant organisms, such as bacteria, into staple crops-regardless of their taxonomic or evolutionary relationship. Through genetic engineering, specific genes can be directly introduced into elite cultivars to enhance nutritional content in two main ways: by modifying nutrient uptake and utilization pathways, and by increasing nutrient bioavailability or reducing anti-nutritional factors (Zhu *et al.*, 2007) [37]. This approach has utilized genes not only from various metabolic pathways found in plants but also from bacteria and other organisms. Although the development of transgenic crops requires substantial investment in the initial stages, it represents a potentially sustainable strategy capable of reaching large populations—particularly in developing countries (Hefferon, 2016) [15].

Targeted vegetables crops for biofortification through genetic engineering

Anthocyanin-rich tomato: To enhance anthocyanin accumulation in the fruits of the commercially cultivated tomato cultivar Arka Vikas, two transcription factors, Ros1 and Del**,** were introduced via Agrobacterium-mediated transformation. Their expression was driven by a fruit-specific promoter to ensure targeted activation during fruit development**.** The resulting transgenic fruits exhibited an average anthocyanin content of 0.1 mg/g fresh weight**,** reflecting 70-100-fold increase compared to the non-transgenic control fruits (Maligeppagol *et al.*, 2013) [20].

Carotenoids-rich tomato: Lycopene, a major carotenoid in tomato, is a potent antioxidant known for its potential to prevent epithelial cancers and promote overall human health. Consequently, there is significant interest in enhancing carotenoid levels in tomato fruit through genetic manipulation. The carotenoid biosynthesis pathway begins with the production of phytoene from geranylgeranyl diphosphate (GGPP**)**, a key step catalyzed by the enzyme phytoene synthase (PSY-1)**.** To increase carotenoid accumulation in tomato fruit, the Psy-1 gene was constitutively expressed, leading to enhanced flux through the carotenoid biosynthetic pathway (Bergougnoux, 2014) [2].

Challenges and limitations of vegetable biofortification: Agronomic and Technical Constraints

Vegetable biofortification whether via breeding or agronomic means depends critically on soil conditions (pH, organic matter, texture) and environmental factors like temperature and moisture. Deficiencies or antagonistic interactions among nutrients (e.g. phosphorus reducing iron or zinc uptake) can greatly reduce efficacy (Ebbisa, 2022) [12]. Moreover, some genotypes may poorly accumulate target micronutrients under given conditions, limiting effectiveness across environments (Marques *et at.*, 2021) [21].

Adding micronutrients via fertilizers (soil or foliar) is sensitive to timing, form of minerals, application method, and local climate. Misapplication can result in environmental pollution, farmer costs, and even crop toxicity. For instance, iodine-applied hydroponically to carrots has led to toxic accumulation; combining multiple nutrients (e.g. Zn and Se) may lead to antagonism, undermining results. Traditional fertilizer-based biofortification is expensive, labor-intensive, and may only benefit farmers able to afford multiple inputs (Ofori *et al.*, 2022) [23].

Breeding approaches depend on genetic variability and often suffer low heritability of traits like iron or zinc content. Generating elite biofortified cultivars requires extensive trials across soils and climates and may take many years. Transgenic strategies can accelerate nutrient introduction, but face regulatory, ecological, and societal hurdles (Priyashantha *et al.*, 2025) [27].

Enhanced micronutrient levels do not necessarily mean improved human nutrition. Antinutrient compounds phytates, polyphenols, tannins, and fiber-bind minerals such as iron and zinc, reducing. Breeding to reduce antinutrients may compromise plant metabolism or stress resistance, while some antinutrients offer health benefits, complicating removal strategies. Balancing promoters (e.g. organic acids, β-carotene) and antinutrients remains a complex task. (Bouis *et al.*, 2010) [6].

Consumer and Farmer Acceptance

Biofortified vegetables may differ in color, flavor, or texture-e.g. orange-fleshed sweet potatoes. In some regions, consumers prefer familiar traits,may reject biofortified variants based on cultural preference or perceived quality differences. Studies indicate that acceptance hinges on preserving sensory characteristics and educating consumers about benefits (https://en.wikipedia.org/wiki/Biofortification?utm_source = www.web).

Farmers do not want adopt novel biofortified varieties if they are not agronomically competitive-i.e. yield, disease resistance, or marketability must match existing cultivars. Without clear advantages and incentives, uptake is limited. Limited seed availability and infrastructure challenges also impede last-mile delivery to smallholder farmers (Marques *et al.*, 2021) [21].

Policy, Regulatory and Economic Limitations

Biofortification, especially genetically modified approaches-must navigate lengthy, inconsistent and often politicized regulatory processes across countries. For example, Golden Rice offers high vitamin A benefits but remains unavailable due to regulatory delays and resistance.

Many programs lack consistent governmental policies to scale biofortified crops. In Ethiopia and Sri Lanka, insufficient funding, weak lab infrastructure, fragmented awareness campaigns, and poor public-private coordination slow progress (Bachewe *et al.*, 2023) [1]. Donor and policymaker interest can wane over time, affecting program sustainability.

Biofortification could create dependence on proprietary seed or fertilizer suppliers, potentially undermining food sovereignty. Critics point out that the approach risks reinforcing reliance on large agritech sectors and oversimplifying dietary systems by focusing on staple crops instead of supporting diverse, holistic food systems. (Malezieux *et al.*, 2024) [19].

Environmental and Sustainability Concerns

Applying mineral fertilizers at scale raises environmental risks-soil degradation, water pollution, algal blooms, and biodiversity loss. Agronomic biofortification thus may conflict with sustainable agriculture aims, unless integrated carefully with ecological practices. Additionally, climate change is causing generalized nutrient declines in staple crops (e.g. α-tocopherol, iron, zinc), which biofortification may only partially offset.

Strategic Gaps and Program Coordination

Program effectiveness depends on coordination across breeding, agronomy, post-harvest handling, extension, and nutrition monitoring. Research from Sri Lanka and Ethiopia emphasizes need for field trials, simplified nutrient testing (e.g. near-infrared spectroscopy), clear incentives, public awareness campaigns, and monitoring frameworks to ensure sustained adoption and nutritional impact. Without multisectoral strategies, biofortification remains siloed and limited in reach (Bachewe *et al.*, 2023) [1].

Conclusion

Biofortification of vegetables presents a sustainable and cost-effective strategy to address micronutrient deficiencies, commonly referred to as 'hidden hunger' especially in populations relying heavily on plant-based diets. Enhancing the nutritional content of vegetables through agronomic practices, conventional breeding, and modern biotechnological tools offers a promising solution to improve public health by increasing the intake of essential nutrients such as iron, zinc, selenium, and iodine. Vegetables are an ideal target for biofortification due to their wide consumption and short growth cycles. Moreover, biofortified vegetables not only provide health benefits to consumers but also improve crop resilience, quality, and market value. However, successful implementation requires collaboration across disciplines, including plant science, nutrition, and policy-making, along with public awareness and acceptance. As global nutritional challenges continue to rise, integrating biofortified vegetables into food systems can play a pivotal role in achieving food and nutritional security in a sustainable and environmentally friendly manner.

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