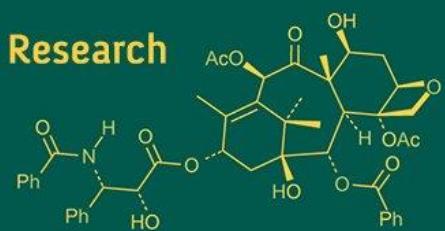
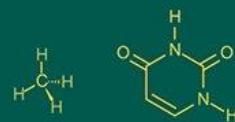


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Biofortification in vegetable crops

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Abstract

Micronutrient malnutrition, also known as "hidden hunger," continues to be a major global public health concern, particularly in developing countries. It affects one-third of the world's population and is caused by inadequate consumption of essential vitamins and minerals such as iron, zinc, iodine, and provitamin A. Increasing the nutritional content of plants using conventional breeding, transgenic techniques, or agronomy methods is known as biofortification, and it has proven to be an economical and long-lasting way to deal with this issue. While staple crops have received most of the focus, horticulture crops like fruits and vegetables have enormous promise because of their natural abundance of vitamins, minerals, and antioxidants. This review discusses recent developments in biofortification techniques like gene editing, molecular breeding, and nanotechnology. Because of their extensive use and natural nutritional diversity, vegetables are perfect candidates for biofortification. With a focus on iron (Fe), zinc (Zn), selenium (Se), provitamin A, and other micronutrients essential for human health, this review summarizes the state of the art regarding vegetable biofortification techniques, advancements, difficulties, and prospects. The method of adding nutrients to food crops, known as "biofortification," offers a long-term, sustainable way to supply more micronutrients. These days, three popular methods are agronomic, conventional, and genetic engineering. Fertilizers used in agronomic biofortification might temporarily raise micronutrient levels. To create plants with the required nutrients, parent lines with high vitamin or mineral levels can be crossed over a number of generations in traditional plant breeding. By transferring desired traits from one organism to another, genetic engineering techniques are used to create new cultivars and increase their value.

Keywords: Micronutrient malnutrition, biofortification, vegetable crops, genetic engineering, nutritional security

Introduction

The term "Biofortification" comes from two words: the Greek word "bio" means "life," and the Latin word "fortificare" means "to make strong." In order to address the detrimental economic and health effects of vitamin and mineral shortages in humans, it refers to the nutrient enrichment of crops as reported by Kumar *et al.* (2025) [20]. Nutrients can be enriched by 1.) Fortification, or adding vitamins and minerals to frequently consumed foods during processing to boost their nutritional worth, is one way to enrich nutrients. 2.) Using vitamin and mineral supplements is known as supplementation. 3.) According to Gomathi *et al.* (2019) [16], Biofortification works better than other techniques for adding nutrients to a crop or plant. When the body lacks the vitamins, minerals, and other nutrients necessary for maintaining healthy tissues and organ function, malnutrition results Cichy *et al.* (2022) [11]. Around the world, malnutrition exacerbates acute anxiety since it causes serious social and health issues as same as A number of strategies aimed at reducing the global burden of disease include nutrition as a key component. The need for higher productivity was met by the green revolution, but quality suffered as a result. The main cause of micronutrient deficiency among the impoverished today is their inability to pay for dietary supplements is similar to Darshan *et al.* (2022) [14]. In both industrialized and developing nations, micronutrient deficiencies particularly those of iron, zinc, vitamin A, selenium, and iodine are serious public health issues. Vegetables are a good source of dietary fiber, antioxidants, and important vitamins and minerals, but unless they are biofortified, their nutritional density frequently falls short of what vulnerable populations need on a daily basis. Different from post-harvest fortification, biofortification is the process of increasing nutrient concentrations in edible plant portions by breeding, agronomy, or biotechnology (Awuchi *et al.* 2020 and

Vandana *et al.* 2022) [3, 30]. The term "bio-fortification" describes genetically boosting food crops' bioavailable mineral content. The development of biofortified crops also increases the crops' growth efficiency on soils that have a depleted or unavailable mineral composition. For crops like vegetables, berries, and melons that have short juvenile periods to reach fruiting stage, breeding plants with increased phytonutrients is easiest Adeyeye *et al.* (2019) [2]. However, it is a much longer-term strategy for tree-fruit and

nuts, which typically require a juvenile period of many years before fruit-set is possible. Finding plant variations with higher phytonutrient content in germplasm collections or current commercial cultivars is one of the alternative approaches Dobosy *et al.* (2020) [15]. This can find lines that consumers might already find acceptable, or it can find a possible donor parent with the right phytonutrient background for transfer to a more acceptable plant-type for consumption.

Table 1: Deficiency of nutrients causes many diseases like due to deficiency of nutrients.

Types of Vitamins	Deficiency Diseases
A (Retinol)	Night blindness
B1 (Thiamine)	Beri-Beri
B2 (Riboflavin)	Retarded growth, bad skin
B12 (Cyanocobalamin)	Anaemia
C (Ascorbic acid)	Scurvy
D (Calciferol)	Rickets
K (Phylloquinone)	Excessive bleeding due to injury
Types of Minerals	Deficiency Diseases
Calcium	Brittle bones, excessive bleeding
Phosphorus	Bad teeth and bones
Iron	Anaemia
Iodine	Goitre, enlarged thyroid gland
Copper	Low appetite, retarded growth

By boosting the nutritional value of crops during their natural growth cycle through conventional breeding, agronomic intervention, or cutting-edge biotechnological techniques, biofortification a relatively new and more sustainable approach avoids these problems (Ofori, *et al.* 2022). Unlike external Review Article fortification or supplementation, biofortified food generates vital micronutrients directly via regular diets without requiring significant behavioural changes or ongoing external input (Martin *et al* 2020) [22]. It is estimated that billions of individuals low intake of key vitamins and minerals like iron, zinc, vitamin A, and iodine, causing weakened immune systems, stunted cognitive growth, excess mortality among children and mothers, and lower economic productivity. This kind of malnutrition, which is characterized by a lack of essential vitamins and minerals despite adequate calorie intake, is particularly common among women and children in developing nations (Parida *et al.*, 2023) [32].

What is Biofortification

The creation of micronutrient-dense staple crops vegetables and grains by the use of current biotechnology, traditional plant breeding techniques, and agronomic methods is known as biofortification. This mechanism increases the concentration of vitamins and nutrients derived from plants in the edible organ as the plant grows and develops O'Hare *et al.* (2015) [31]. This method of breeding nutrients in food crops offers a long-term, affordable, and sustainable supply of sufficient micronutrients. It involves using the proper breeding strategy and biotechnological techniques to enrich edible parts such as grain, straw, roots, fruits, and tubers with minerals and vitamins by Saltzman *et al.* (2013) [26]. By enhancing the daily adequacy of micronutrient intake by the individual throughout the life cycle, biofortified staple foods can help minimize "hidden hunger" even if they may not contain as many critical vitamins and micronutrients as industrially fortified meals Cichy *et al.* (2022) [11]. Agronomic methods, traditional breeding methods, and genetic engineering methods are all used in biofortification.

Additionally, biofortification offers a potential way to spread food and technology to a population that is undernourished and may not have easy access to a variety of diets. 5 Biofortification of Vegetables 107 conventionally fortified foods and supplements Ofori *et al.* (2022). The various forms of biofortification can greatly raise the amount of vitamins and nutrients in the living product edible portion, and the accumulation of these elements occurs as a result of the plant's regular physiological processes Atulkunda *et al.* (2021) [4]. The low-cost solution for underdeveloped nations and their citizens who cannot afford high-quality food is to breed plants to increase the Bioavailability of micronutrients in the desired edible food. This strategy will therefore assure improved health by mitigating micronutrient deficiency. The improvement of vitamins and micronutrients in staple foods and vegetables will increase micronutrient consumption, especially among the impoverished, which would ultimately result in a decrease in malnutrition Afify *et al.* (2023) [1]. Increasing phytonutrients and vitamins in a plant with a lesser life cycle which have short juvenile period to reach the flowering stage like in vegetable can be easily achieved. But this will be difficult for the tree-fruit and nuts where the juvenile period is much longer Das *et al.* (2017) [12]. The principal objective of biofortification in vegetables should be the production of the nutritionally enriched vegetable crop so that the negative economic and health consequences can be overcome by managing mineral and vitamin deficiencies in humans. Among crops cultivated the fruits and vegetables have a rich source of genetic diversity for micronutrients, and hence they can be easily biofortified through conventional breeding, marker-assisted breeding, or genetic engineering Dwivedi *et al.* (2017). Strategies for biofortification of vegetables with the enhancement of mineral and vitamin, reduction of anti-nutrients to increase the bioavailability of micronutrients.

Concept of Biofortification: Biofortification refers to the enhancement of nutrient concentration and bioavailability in

edible plant parts. Unlike food fortification, biofortification targets the crop itself, ensuring long-term nutritional benefits, especially for rural populations with limited access to diverse diets Ikram *et al.* (2025) [18]. Advances in genomics, marker-assisted selection, and gene-editing tools such as CRISPR/Cas9 offer new opportunities to accelerate vegetable biofortification. Integration of agronomic and genetic approaches, along with nutrition education, will enhance the impact of biofortified vegetables Cichy *et al.* (2022) [11].

Importance of Biofortification

In addition to providing naturally fortified foods to populations with limited access to commercially marketed fortified foods, biofortification offers a very affordable,

sustainable, and long-term way to supply more micronutrients in relatively remote rural locations (Roriz *et al.*, 2020) [25]. Biofortified staple foods can aid by raising the daily adequacy of micronutrient intakes among individuals throughout their lifecycle, but they cannot provide as high a daily level of minerals and vitamins as supplements or industrially fortified meals Rana *et al.* (2019). It is not anticipated that biofortification will cure or eradicate micronutrient deficiencies in every demographic. The issue of micronutrient deficiency cannot be resolved by a single intervention, but biofortification works in tandem with current strategies to sustainably supply micronutrients to the most vulnerable individuals in a reasonably priced and economical manner Tengali *et al.* (2021).

Table 2: Sources of nutrients from vegetables

Nutrients	Vegetables
Carbohydrate	Sweet potato, potato, cassava
Protein	Pea, lima bean, French bean, cowpea
Vitamin A	Carrot, spinach, pumpkin
Vitamin B1	Tomato, chilli, garlic, leek, pea
Vitamin C	Chilli, sweet pepper, cabbage, drumstick
Calcium	Hyacinth bean, amaranthus, palak
Iron	Amaranthus, palak, spinach, lettuce, bitter gourd
Phosphorous	Pea, lima bean, taro, drumstick leaves
Vitamin B5	Palak, amaranthus, bitter gourd, pointed gourd
Iodine	Tomato, sweet pepper, carrot, garlic, okra
Sodium	Celery, green onion, Chinese cabbage, radish

Nano micronutrients are essential plant nutrients, such as iron, zinc, and manganese, encapsulated or formulated into nanoparticles to improve their delivery and absorption by plants. This nanotechnology enhances nutrient use efficiency by enabling slower, controlled release into the soil or to the leaves, preventing over-fertilization and reducing costs for farmers while promoting agricultural sustainability and increased crop productivity and quality Choudhary *et al.* (2020) [10]. They exhibit significant catalytic activity and a big surface area in relation to their size. Essential plant nutrients including iron, zinc, and manganese are known as nano micronutrients. These nutrients are encapsulated or synthesized into nanoparticles to enhance plant absorption and delivery (AOAC 2026) [6]. Additionally, the term "nano-PGPR" refers to Plant Growth-Promoting Rhizobacteria (PGPR) that have been nano-encapsulated or nano-formulated. This technique employs nanotechnology to increase the efficiency of helpful soil bacteria in agriculture Sharma *et al.* (2020) [29].

Methods of Biofortification

Biofortification can be achieved through three strategies

- Agronomic Biofortification
- Conventional plant breeding
- Genetic engineering

Agronomic Biofortification

Application of fertilizers to boost the amount of micronutrients in edible portions. The best micronutrients for agronomic biofortification are selenium (as selenate), zinc (foliar treatments of ZnSO₄), and iodine (soil application of iodide or iodate) Roriz *et al.* (2020) [25]. The quick and simple way to fortify micronutrients (Fe, Zn, Cu, etc.) in plants is through foliar spray. Mycorrhizal

relationships raise the concentrations of Fe, Se, Zn, and Cu in crop plants, according to several studies. Micronutrients such as Zn, Cu, and Fe are more efficiently absorbed when AM-fungi are present. Onion sulphur concentration is increased by sulphur-oxidizing bacteria Dobosy *et al.* (2020) [15].

Biofortification of crops with Iron

The production of hemoglobin and myoglobin, which are involved in the movement of oxygen in blood and muscles, depends on iron. Anemia, frailty, stunted child growth, and increased maternal mortality are all consequences of iron deficiency Jangra *et al.* (2025) [19]. High-iron beans (*Phaseolus vulgaris*), specifically cultivated through traditional breeding methods in East Africa, are a hallmark of biofortified crops for iron (Beebe, 2020) [9]. Due to its inherent high iron content, spinach and amaranth have also been selected to have their iron content increased through variations. Iron-rich leafy greens that can be further improved are pumpkin and cowpea leaves. He also stated that *Spirulina platensis* has been employed as a biofortifying agent to improve the iron status in *Amaranthus gangeticus* plants, and that utilizing *S. platensis* as a microbial inoculant increased the iron levels of *Amaranthus* plants when compared with control Smolen *et al.* (2019) [32].

Biofortification of crops with Zinc

At a foliar Zn treatment rate of 1.08 g plant-1, the association between tuber Zn content and foliar Zn application followed a saturation curve, peaking at around 30 mg Zn kg⁻¹ DM Gomathi *et al.* (2017) [16]. Even after foliar Zn fertilization with 2.16 g Zn plant-1, the content of Zn in the shoots increased by 40 times compared to the unfertilized controls. The application of "Riverm" fertilizer aids in the zinc enrichment of tomatoes, eggplant, and sweet

peppers. Vegetables that have been biofortified had 6.60-8.59% more zinc than control Rahim *et al.* (2020) [24].

Biofortification of crops with Selenium

When used as a soil amendment, Se-enriched *S. pinnata* provides beneficial forms of organic-Se to broccoli and carrots Kalia *et al.* (2017) [21]. A 77Se (IV) solution that was enriched to 99.7% as 77Se was applied topically to onions and carrots to biofortify them. Selenium treatment had no effect on the production or oil content of brassica crops Adeyeye *et al.* (2019) [2]. There found a high concentration of Se (1.92-1.96 µg Se g⁻¹) in the meal and seeds.

Conventional plant breeding

The amount of nutrient status in the current varieties has dropped due to traditional breeding's emphasis on yield qualities and resistance breeding during the past 40 years. Fe, Zn, Cu, and Mg are a few examples of minerals whose mean concentration in the dry matter has decreased in a number of plant-based meals (Athar *et al.*, 2020) [5]. Fortification of critical vitamins, antioxidants, and micronutrients has been emphasized in recent developments in conventional plant breeding. Adequate genetic variation in concentrations of β-carotene, other functional carotenoids, iron, zinc, and other minerals must exist among cultivars in order for conventional breeding to increase the micronutrient density of staple foods Dwivedi *et al.* (2023) [13]. This allows for the selection of nutritionally appropriate breeding materials.

Biofortification through Genetic Engineering

The direct use of biotechnology to alter an organism's genes is known as genetic engineering or genetic modification. By transferring desirable traits from one organism to another, genetic engineering makes it possible to create new cultivars that are elite and significantly increase their value (Athar *et al.*, 2020) [5]. It has the potential to improve edible crops' nutritional content and bioavailability. Additionally, it presents special chances to enhance nutritional quality and provide additional health advantages. Numerous vegetable crops have undergone genetic modification to enhance characteristics like improved flavor or nutritional status, as well as to decrease bitterness, slow ripening, greater nutritional status, seedless fruit, increased sweetness, and anti-nutritional effects (Bouis and Saltzman, 2017) [7].

Conclusion

By distributing naturally fortified foods to those with limited access to commercially promoted fortified foods, which are more easily accessible in metropolitan areas, biofortification offers a practical way to address malnourished populations in relatively remote rural locations. Therefore, commercial fortification and biofortification are quite complimentary. Adequate intakes of a variety of nutrients and other substances, in combinations and amounts that are yet unclear, are ultimately necessary for healthy nutrition. Therefore, increasing the consumption of a variety of non-staple foods is the best and last way to end undernutrition as a public health issue in developing nations. To achieve this, though, it will need several decades, well-informed government policies, and a large investment in agricultural research as well as other public and on-farm infrastructure. In summary, according to M.S. Swaminathan, "GM foods have the potential to solve many of the world's hunger and

malnutrition problems, and to help protect and preserve the environment by increasing yield, quality, and reducing reliance upon chemical pesticides." However, governments still face numerous obstacles, particularly in the fields of industrial policy, regulation, safety testing, and food labelling.

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