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Biofortification: An encouraging strategy for enhancing micronutrient levels in vegetable crops-A review

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Abstract
By 2030, the goal is to eliminate hunger and guarantee that all people, especially the poor and vulnerable, including infants, have consistent access to safe, nutritious, and adequate food throughout the year. This objective is part of the United Nations Sustainable Development Goal (SDG) 2, "Zero Hunger," one of 17 global goals aimed at eradicating poverty. Key measures of success for SDG 2 include reducing undernourishment and addressing moderate to severe food insecurity. Historically, food security was the primary global concern, but with advancements in this area, developing nations are now shifting their focus to nutritional security, which emphasizes foods rich in essential vitamins and minerals. Micronutrients like iron, zinc, selenium, magnesium, calcium, iodine, and vitamins such as provitamin A and folate are crucial for human growth and development. A deficiency in these nutrients results in what is known as "hidden hunger." Addressing these deficiencies through biofortification can help alleviate malnutrition, particularly among women and children in developing countries. The biofortification of vegetables with vitamins and minerals are now an urgent need to combat various health challenges. Three main methods are used for biofortifying vegetables and staple crops: conventional breeding, the agronomic approach (applying mineral fertilizers), and genetic engineering. These strategies hold great potential to reduce vitamin and micronutrient deficiencies, and a variety of genes can be targeted to improve micronutrient content in vegetables. These tools can significantly boost the levels of vitamins and micronutrients in staple crops and vegetables, offering a way to tackle malnutrition.

Keywords: Biofortification, vegetables, iron, zinc, iodine, selenium.

Introduction
The United Nations Sustainable Development Goal (SDG) 2, "Zero Hunger," is one of 17 global targets aimed at eradicating poverty. Key indicators for achieving this goal include reducing undernourishment and tackling moderate to severe food insecurity (Kropff 2018) [16]. Nutritional deficiencies remain a widespread issue in many developing countries, driven by limited resources and growing populations. The lack of adequate micronutrient intake in diets leads to malnutrition, which negatively impacts global health. Several strategies have been implemented to increase the availability of micronutrients in diets, as nutritional inadequacy not only affects public health but also has significant economic consequences (Monika *et al.*, 2023) [18]. The annual economic burden from deficiencies in micronutrients and macronutrients is estimated at $2 trillion USD. Therefore, improving nutrition is a critical priority for many countries. Climate change poses additional challenges, as varying climate conditions lead to environmental disasters. Around 1.8 billion people live in areas with severe water stress, and this number is projected to rise to 2 billion by 2025. Water scarcity affects both food security and crop production. To combat malnutrition, biofortification has emerged as a sustainable, cost-effective method for increasing micronutrient availability in populations with limited access to diverse foods and other nutritional interventions (Bouis and Saltzman 2017) [3]. This long-term strategy improves mineral nutrition by enhancing the mineral content and bioavailability in staple crops (Monika *et al.*, 2023) [18]. Biofortification involves increasing the concentration of vitamins and minerals in crops through plant breeding, genetic modification, or agronomic practices. Regular consumption of biofortified crops can lead to measurable improvements in health and nutrition.

Important Terms associated with biofortification

"Malnutrition" refers to a deficiency, excess, or imbalance of various nutrients that significantly impacts body composition, function, and overall health outcomes. The term "Hunger" is commonly used in social science and policy discussions to describe individuals who suffer from a prolonged lack of adequate food, often experiencing persistent or frequent feelings of hunger. In a literal sense, it refers to the body's physiological need for food (Jana 2022) [13]. "Hidden hunger" is a nutritional issue that arises from both undernutrition and overnutrition.

Micronutrients are, so important? Why?

The growing global population presents a serious threat to nutritional security, requiring the adoption of practical and affordable measures within the global food system. Over 2 billion people worldwide face deficiencies in essential micronutrients such as iron (Fe) and zinc (Zn). Additionally, micronutrient deficiencies in soil have been observed in many regions, limiting nutrient absorption by plants and, consequently, by humans. These micronutrients are crucial for proper bodily functions, and their inadequate intake leads to significant health consequences. Moreover, the demand for livestock feed far exceeds the available supply, particularly due to insufficient and low-quality fodder production. Plant-based foods constitute a large part of human diets, providing essential calories, nutrients, and bioactive compounds needed to maintain good health and prevent diseases. Vegetables, in particular, are key components of a plant-based diet, offering dietary fibre, phytochemicals (like vitamins and antioxidants), and minerals. Minerals, which are inorganic elements needed for biological functions, are required in specific amounts of the 17 essential minerals, 11 are generally abundant in food and water, making deficiencies rare in all but extreme cases. However, iodine (I), iron (Fe), zinc (Zn), calcium (Ca), and selenium (Se) are less commonly found in sufficient quantities in some foods. Diets dominated by staples with low mineral content, such as milled grains, are prone to deficiencies (Christou and Twyman 2004) [7]. Mineral deficiencies are more frequent in developing countries, where fresh produce is scarce, but calcium deficiencies also affect developed nations (Galera *et al*. 2010) [10]. Iodine is essential for producing thyroid hormones that regulate growth, development, and metabolism. Only about 30% of the body's iodine is stored in the thyroid gland, while the function of the other 70% remains unclear. Iodine deficiency can lead to goitre, which results from the thyroid gland enlarging due to insufficient thyroxine production (Dunn 2003) [8]. Iron is vital for oxygen transport and energy metabolism, acting as the functional core of haemoglobin and myoglobin. It also plays a role in the catalytic activity of non-hemi enzymes. Iron deficiency anaemia (IDA), affecting over two billion people worldwide, is a direct result of insufficient iron intake. More than half of these cases could be resolved by improving dietary iron intake, but this is challenging in developing countries where staple foods, like grains, have low iron content and contain compounds like phytate that hinder iron absorption. Zinc is involved in numerous bodily functions, acting as a cofactor for around 100 enzymes and as a signalling molecule in the immune and nervous systems. Zinc deficiency, which affects nearly two billion people, particularly children and pregnant women, can cause hair loss, skin issues, growth delays, and developmental problems. Calcium, the most abundant mineral in the human body, is essential for bone structure, blood clotting, and as an enzyme cofactor. Calcium deficiency can lead to bone diseases such as rickets in children and osteoporosis in adults. Selenium is another essential mineral that plays a role in about 50 enzymes, including those

Table 1: Major Minerals present in Vegetables

S. No	Nutrition	Vegetables
1	Vitamin A	Bathua, Colocasia Leaves, Carrot, Spinach, Pumpkin, Sweet Potato, Cassava
2	Vitamin B1	Chilli, Tomato, Garlic, Leek, Pea
3	Vitamin B2	Fenugreek
4	Vitamin B5	Palak, amaranthus, bitter gourd, pointed gourd
5	Vitamin C	Parsley, drumstick, coriander leaves, chilli, capsicum, cabbage
6	Carbohydrates	Tapioca, Sweet Potato, Potato
7	Protein	Lima Bean, Peas, Cowpea, French Bean
8	Fat	Potato
9	Calcium	Agathi, curry leaves, hyacinth bean, amaranthus, palak
10	Phosphorus	Amaranthus, garlic, pea, lima bean, taro, drumstick leaves
11	Iron	Amaranthus, Palak, spinach, lettuce, bitter gourd
12	Zinc	Spinach, Broccoli, Kale, Garlic
13	Potassium	Spinach, Amaranthus, Cucumber
14	Calories	Tapioca, Garlic, Lima Beans
15	Iodine	Tomato, Capsicum, Carrot, Garlic, Okra
16	Sodium	Celery, green onion, chinese cabbage, radish

Involved in reducing oxidative stress and regulating thyroid hormones (Lyons *et al*. 2004) [17]. Folate deficiency, associated with increased risks of maternal death and birth complications, can be addressed through the fortification of wheat products. Vitamin A deficiency affects around 140 million children and 7 million pregnant women, particularly in Africa and South/Southeast Asia, leading to blindness and increased risk of disease and mortality. Vitamin A supplementation programs have been introduced in over 40 countries to combat this issue (Haddad *et al*. 2004) [12].

The importance of bio-fortification

The Green Revolution and similar movements in India were primarily aimed at eliminating hunger across the country. As a result, India has significantly increased its food grain production and is now mostly self-sufficient. Various government schemes and initiatives have been implemented to ensure that the population receives adequate food in terms of caloric intake. However, the current emphasis is on enhancing the nutritional content of the food consumed. Although there is "enough to eat," many individuals still lack essential nutrients in their diet, leading to the issue of "hidden hunger" (John and Babu 2021) [14]. Improving the nutritional quality of crops is crucial for enhancing daily diets, eradicating malnutrition, and strengthening food security. In the long term, biofortified crops can also improve farmers' incomes, particularly benefiting rural communities with limited access to diverse diets, fortified foods, or supplements (Chaudhary *et al*. 2022) [6].

Table 2: Nutraceutical values of vegetables reference (Gluastus Horticulture book 1st edition)

Crop	Botanical Name	Nutraceuticals	Disease Control Potential
Legume Vegetables	*Pisum sativum, Vigna Unguiculata, Cyamopsis tetragonal*	Isoflavonoids	Osteoporosis, Obesity &Menopause
Okra	*Abelmoschus esculentus*	Quercetin & flavanol derivatives	Diabetes & Vitality
Potato	*Solanum tuberosum*	Lysine, Chlorogenic acid	Cold stores & BP
Brinjal	*Solanum melangena*	Caffeic acid, Chlorogenic acid & Nasunin	CVD Skin blemishes
Broccoli	*Brassica oleracea var. italic*	Glucobrassicin, Progoitrin & Gluconasturtin	Cancer
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Artichoke, Amaranth us	*scolymus Amranthus spp*		
Spinach Fenugreek	*Spinacea oleracea Trigonella foenum-graceum*	Vitamin E, C & Folates	CVD & Constipation
Onion Gralic	*Allium cepa, Allium sativum*	Allyl propyl disulphide, Quercetin, Allin & Methionine	Stomach & colon cancer, cough, cold, diabetes, hypertension
Carrot Pumpkin Cantaloupe	*Daucus carota, Cucurbita moschata Cucumis melo var. cantalupensis*	Vitamin A	Bladder cancer & Lymphoma
Endive, Horse Radish	*Cichorium intybus var. folisum, Armoracia rusticana*	Kaemphoral, Myricetin & Fisetin	Inhibit LDL, Antioxidant, Anti carcinogenic
Celery Broccoli	*Apium graveolens Brassica oleracea var. italica*	Luteolin, Apigenin	Cancer of breast, Skin

Techniques of Biofortification

Agronomic practices

Agronomic biofortification involves applying fertilizers to enhance the concentration of micronutrients in crops grown in soils that are deficient in these essential minerals (Rengel *et al*. 1999) [23]. This process can be achieved by adding micronutrient-enriched fertilizers, which is a quick and effective way to improve the nutritional content of crops. Consuming these biofortified crops can, in turn, enhance human nutrition. Agronomic biofortification generally focuses on nutrient application, solubilization of minerals, and the transfer of nutrients from the source to the edible parts of the plant. Key macronutrients such as nitrogen (N), phosphorus (P), and potassium (K) play a crucial role in achieving higher yields. During the 1960s, the push to produce more macronutrients resulted in a significant rise in agricultural yields, contributing to the Green Revolution, which helped prevent widespread hunger, particularly in developing countries.

Today, with the need to feed a growing global population of around seven billion people (Graham *et al*., 2007) [11], the goal is not only to produce more food from limited resources but also to enhance the micronutrient content in the edible parts of plants for better health. Micronutrients are absorbed from the soil and present in varying amounts in different parts of the plant. The use of micronutrient-enriched fertilizers can improve both soil quality and plant nutritional value, helping to address deficiencies in both plants and humans. However, these micronutrients often become immobilized in the soil and are not easily transported to the edible portions of the plant. To overcome this, alternative methods like foliar sprays of soluble micronutrients are recommended. Agronomic biofortification is a practical and cost-effective strategy when factors such as fertilizer type, method of application, and timing are taken into account.

Conventional plant breeding

Traditional breeding methods involve creating sufficient genetic variation to achieve desired traits in crops, such as high levels of specific micronutrients. This process involves crossbreeding different plant varieties over several generations to ultimately develop a plant that not only has a higher nutrient content but also possesses other favorable traits (Rengel *et al*. 1999) [23]. In India, this is the primary method used to produce biofortified crops. Over the past decade, traditional breeding has become a widely adopted, cost-effective, and practical approach to fortifying crops with micronutrients (Van Der Straeten *et al*., 2020) [25]. The success of this technique depends on the presence of enough genetic variation in the traits being targeted. Existing crop varieties with high levels of vitamins and minerals are used as parent lines and are crossed with other varieties that have desirable agricultural characteristics. This breeding continues over multiple generations to produce plants with both high nutrient content and favorable agronomic traits. A significant challenge in this method is the lack of genetic diversity in the gene pool, which can limit the development of desired traits. This can be addressed by crossing with distant relatives, although this may reduce the transmission of desirable traits into the commercial crop. Mutagens can also be used to develop commercial varieties. Given the effectiveness of breeding to improve plant characteristics, numerous organizations worldwide have initiated breeding programs to enhance the micronutrient content of crops.

Genetic engineering/modification

Genetic engineering is a cutting-edge approach to combating mineral deficiencies, using advanced biotechnological techniques to directly introduce specific genes into crop varieties. These genes, which can come from various sources such as animals or microbes, aim to achieve objectives like improving the efficiency of mineral uptake from soil, reducing antinutritional compounds, or increasing nutritional enhancers like inulin (Zhu *et al*. 2007) [29]. This process involves inserting DNA into an organism's genome to introduce new traits, such as disease resistance (Rengel *et al*. 1999) [23]. Genetic engineering, also known as recombinant DNA (rDNA) technology, allows for the precise transfer of specific genes from an unrelated source organism directly into the target organism's DNA. This method, which bypasses the natural reproduction process, enables traits to be passed down through normal reproduction once integrated into the plant. The technology was first developed in 1973 and has been applied to modify agricultural crops since the 1980s. After identifying a suitable gene-an often time-consuming and labour-intensive process-it is combined with marker and promoter genes and introduced into a plant, usually via a nonviable virus like *Agrobacterium*, acting as a carrier. While traditional plant breeding is less resource-intensive and faces fewer regulatory challenges compared to genetic engineering or marker-assisted selection, it has limitations. For instance, if a crop lacks genes for specific traits like vitamin A precursors, no amount of traditional breeding can introduce them, making genetic engineering necessary. Molecular breeding and genetic engineering offer advantages over traditional methods, such as enabling the development of crops with multiple desired nutritional traits, ensuring the agronomic viability of biofortified crops, and adapting improvements made in developed nations for crops grown in developing countries (Zhu *et al*. 2007) [29]. Unlike traditional breeding, genetic engineering allows for the transfer of heritable traits between entirely unrelated species, creating combinations of traits that could not be achieved through conventional methods. Several factors contribute to the successful genetic transformation of crops, including the development of reliable tissue culture methods, the construction of gene constructs with appropriate promoters, efficient transformation techniques, and the characterization and transfer of transgenes into elite cultivars using conventional breeding techniques.

Mutation breeding

Mutation breeding has been extensively utilized in both developed and developing nations to create grain varieties with enhanced quality, yield, and other traits. This method leverages the increased genetic variability that results from chemical treatments or irradiation. The FAO/International Atomic Energy Agency (IAEA) maintains a database that lists over 2,500 varieties produced through mutation breeding. Among these, 1,568 are from Asia, 695 from Europe, and 165 from the United States. While most mutants in Europe and America are flowers, the majority of Asian mutants consist of staple food crops such as wheat, rice, maize, and soybeans. The FAO/IAEA recognizes biofortification as a goal within their mutagenesis program, although no relevant data has yet been published. In the United States, crops developed through mutagenesis can be grown and certified as organic, unlike transgenic crops created using recombinant DNA (rDNA) technology.

Molecular Breeding

Molecular breeding, also known as marker-assisted breeding, is a powerful modern biotechnology tool that even organic farmers have begun to adopt because it alters plant DNA using biological breeding methods instead of engineered gene insertions. Advances in genomics-the study of gene locations and functions-and the significant reduction in costs for plant tissue screening have accelerated the development of this technology. After locating a gene associated with a desired trait, scientists create a probe that binds to a specific DNA fragment, called a marker, unique to that gene. This marker can then be used to track and expedite the introduction of this trait into related plant species through traditional breeding methods. Since markers can be identified in the tissues of young seedlings, the presence or absence of the desired trait can be determined without waiting for the plant to mature, potentially shortening the typical growth cycle by several years (Pray 2006) [20].

Biofortification Application Challenges in India

In India, there are several challenges associated with biofortification and the integration of biofortified food grains into daily diets. These challenges include changes in grain color, which can lead to reluctance among consumers to accept biofortified foods like golden rice. Additionally, widespread adoption by farmers is necessary, but initial costs may pose a barrier to implementation (Durga Bhavani 2021) [9].

Future Challenges

Future efforts should focus on producing crops with higher iron concentrations for human nutrition. It will also be important to explore biofortification methods beyond simply reducing phytic acid or polyphenol levels to enhance iron absorption without compromising their beneficial properties. Moreover, crops that overexpress ferritin should be evaluated for potential heavy metal accumulation before being released to the public.

Conclusion

Achieving the United Nations Sustainable Development Goal of "Zero Hunger" necessitates a multifaceted approach to combat malnutrition and food insecurity, particularly in developing countries. Biofortification stands out as a sustainable and effective strategy to enhance the micronutrient content of staple crops, addressing the pervasive issue of hidden hunger. By improving the nutritional quality of food, biofortification not only supports public health but also contributes to economic stability, especially in rural communities. However, challenges such as consumer acceptance and initial costs must be addressed to ensure widespread adoption. As climate change exacerbates food security issues, prioritizing innovative agricultural practices will be essential for fostering resilient food systems and improving global nutrition.

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