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Biofortification of vegetable crops to boost nutraceuticals in human diet: A Review Short running title: Biofortification approaches for vegetable crops

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ABSTRACT

Nutritional security is the primary subject of study for developing nations after food security. By biofortifying grains and vegetables, agricultural scientists can alter the physiology of crops and fight "hidden hunger," which affects the majority of people in underdeveloped nations. Plant breeders, molecular scientists, and genetic engineers have a lot of opportunities to increase the micronutrient density and vitamin content of vegetables and staple food crops for underdeveloped nations. Vegetables have sufficient genetic variability, but there are still a few characteristics that need to be looked at. The current study focused on the biofortification of vegetable crops through the use of agronomic, conventional and genetic engineering approaches, because the health of individuals is seriously affected due to the poor intake of nutrients. Both traditional breeding and genetic engineering call for the introduction of certain features when used to boost micronutrients and vitamin content. Recent genetic discoveries have made it feasible to increase micronutrients by decreasing anti-nutrients like phytic acid or tannins. Vegetables may be biofortified using genome editing techniques like ZFN, TALENS, CRISPR-Cas9, etc. that can alter plant genes or knock down undesired characteristics. Many methods for achieving desired genetic change without the regulatory difficulties associated with transgenic technology are now possible due to recent advances in genome editing. The goal of current research is now to encourage a healthy lifestyle via the use of dietary supplements and a diet rich in fruits and vegetables.

Keywords: *biofortified, genetic engineering, genetic variability, genome editing, micronutrient, nutritional security, vegetables*

Introduction

The terms "biofortification" and "biological fortification" relate to food crops that were developed and cultivated utilizing contemporary biotechnology methods, traditional plant breeding, and agronomic practices and are nutritionally enriched with greater bioavailability for the human population. The process of biofortification involves combining conventional plant breeding techniques with cutting-edge biotechnology and agronomical strategies to produce staple foods (cereals and vegetables) that are high in micronutrients [43]. Several dietary recommendations for human health and disease prevention have emphasized eating habits based on an increase in the consumption of fresh produce and a decrease in the consumption of salt, simple carbohydrates, and saturated fats [67]. People need a variety of mineral minerals, which must be present in their diet, to sustain optimum health. Because certain minerals are required for numerous physicochemical processes and cannot be absorbed alone or function without them, this shows the importance of minerals [25].

All essential minerals are found in food, primarily plant-based food, thus it's crucial to regularly consume a good, balanced diet that can offer an acceptable proportion of minerals. Yet, adding healthy substances and minerals to meals could be seen as a tactic to address specific nutritional needs or combat undernourishment [65]. Moreover, worries about overnutrition are spreading. Increased crop and grain productivity has traditionally taken precedence in our agricultural economy above safeguarding public health. This process has resulted in dietary grains having much less micronutrient content than they had before, which has exacerbated the consumer micronutrient deficit. Nowadays, agriculture is moving away from cultivating enough nutrient-rich crops and towards growing more food crops in larger numbers. This will help fight "hidden hunger" and "micronutrient malnutrition," especially in developing and impoverished areas where the bulk of meals consist of staple foods lacking in micronutrients [29]. Nutrient supplementation programs have historically provided minerals and vitamins to the general people, but this approach falls short of the objectives stated by international health organizations since it depends on financing that may change from year to year. Moreover, hurdles include low-income individuals' limited buying power, access to markets and healthcare systems, and knowledge of the long-term health advantages of taking vitamin supplements [48]. Hence, biofortifying a range of crop kinds is a long-term, sustainable way to guarantee that people have access to meals high in micronutrients. Additionally, clients may get biofortified crops through conventional methods of food production and distribution, which have higher bioavailable concentrations of vital micronutrients. This is a practical strategy to assist undernourished low-income families that have little access to a variety of dietary options, supplements, and fortified foods. From an economic standpoint, biofortification is a one-time investment that offers a long-term, sustainable solution to the issue of hidden hunger [27, 37, 42, 49, 52].

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In emerging nations, there may be a considerable rise in population over the next decades. Together with a changing climate, this might make ensuring food security more difficult [3, 12]. Thus, one of the key objectives of organizations like the World Health Organization and the Consultative Group on International Agricultural Research (CGIAR) is the creation of nutritionally improved high-yielding biofortified crops [5].

Health benefits of biofortified vegetables

Plant foods, which comprise a significant amount of the normal person's diet, contain the bulk of the calories, minerals, and bioactive compounds needed to sustain health and ward off disease. Vegetables, which contain dietary fiber, phytochemicals, and minerals, are a staple of a plant-based diet [18, 66]. Minerals must be obtained through diet because humans are unable to manufacture them. Minerals are thought to be essential nutrients. The diet (Figure 1) that allowed humans to evolve was mostly dependent on vegetables, and a diet deficient in them is one of the main causes of many non-communicable diseases that are common in Westernized nations. Minerals and iodine, for instance, can be found in a diet high in vegetables and assist in maintaining normal blood pressure, bone strength, hormone production, heart health, and mental wellness [17].

Vegetables play a major role in combating poverty and malnutrition [8, 44]. The micronutrients must be freed from the food matrix, absorbed into the blood, and transported to the intended tissues along the gastrointestinal tract's passage [4]. The fraction that is released from the plant tissue is the only part that eventually becomes absorbable. Agronomic techniques to boost plant phytochemicals and mineral bioaccessibility are promising targets to improve the nutritional value of vegetables [10]. Vegetable intake should increase in the upcoming years due to reasons related to health and the environment.

Approaches for biofortification in vegetable crops

Several strategies are used to alleviate micronutrient malnutrition; the most popular ones are product fortification and medical supplementation. Fortification is the practice of adding nutrients to food while processing it using various techniques. Fortification, meanwhile, can be difficult in some situations because of subpar investments, infrastructure, and distribution methods. An alternate course of action in these circumstances is to adopt novel genotypes with enhanced compositional profiles or to customize particular agronomic approaches intended to increase the content of particular health-effective chemicals in common crops [23]. While this may be a possibility for items that are altered before consumption (such as staple meals), biofortification is the only way to increase the amount of health-promoting substances in the edible section of products like fresh vegetables.

Increasing the number of minerals or other particular health-related ingredients in the edible portion is the aim. Through the genetic alteration of a species, a transgenic program permits the production of plants with desired features (e.g., higher content of specific nutrients). This methodology is now the least employed due to the highly slow and expensive research and development procedure, even though there may be long-term cost savings possibilities. Regardless, the higher costs connected with producing biofortified vegetables are offset in developed countries by the creation of a quality product with higher nutritional content and with the ability to satisfy the growing demand from customers willing to spend more for healthier food [62]. Furthermore, several nations have various rules that prevent the use of GMOs (genetically modified organisms).

Biofortification can be achieved through three strategies (Figure 2):

1. Agronomic approaches
2. Conventional plant breeding approaches
3. Genetic engineering approaches

Agronomic approaches

It is one of the most straightforward techniques of biofortification of vegetables. This approach, however, is time and resource-intensive and only works effectively in nations where the genetic engineering-based biofortification method is not widely adopted. With this method, fertilizer is often applied as a soil addition or as a leaf spray. Fe and Zn were successfully biofortified in plant tissue and edible components by using a foliar spray to boost these nutrients [57]. For biofortification, the agronomic strategy also comprises methods for controlling the crop-growing season. Micronutrients are improved by the combination of practices, including water management, nutrient interaction, and tillage. Foliar application over soil application is favored for agronomic biofortification since it uses less Fe and Zn fertilizer [51].

Hydroponic system farming is possible to develop in areas with inadequate agricultural soil and make the most of limited water resources thus, soilless agriculture techniques have gained popularity. Studies have shown that one of the best methods for increasing the nutritional content of plant tissues is occasionally to use hydroponic cultures [32, 71]. Programs for biofortification often include the addition of selenium since it is a trace element that has a significant influence on how plants use antioxidants and because it accumulates poorly in species like *Solanum lycopersicon*. In a study, five sodium selenite (Na_2SeO_3) dosages of selenium (0, 2, 4, 6, and 8 mg L^{-1}) were added to a nutritive solution in a hydroponic system. The obtained results demonstrated that agricultural biofortification with Se given in the nutritional solution enhanced tomato fruit Se concentration, yield, and nutraceutical quality [21]. It has been reported that Spirulina platensis has been employed as a biofortifying agent to boost the iron status in *Amaranthus gangeticus* plants by increasing iron levels of amaranthus plants by employing it as a microbial inoculant when compared to control [1]. The examples of biofortified elements and varieties developed in various vegetable crops are given in Table 1 and Table 2.

Conventional plant breeding approaches

Most of the methods for increasing the amount of vitamins and minerals in the plant's edible section are yet unknown. More fundamental research is required to gather micronutrients, synthesize micronutrients in usable forms, and increase the vitamin content of seeds, grains, tubers, and other edible components of popular cereals and vegetables. Using genetic engineering, traditional breeding methods, and other procedures, certain crops may be biofortified and spread globally [68]. This time, the focus of the research is on developing biofortified crops. When the plant has been developed and acquired the farmer's permission, the seed may be multiplied, duplicated, and distributed among all farmer groups in the specified region. In the end, this will support the crop's exceptional nutritional properties throughout time [24]. Traditional breeding is based on natural diversity, making it a potential replacement for genetic engineering research. It was discovered that newly developed breeding lines for crops like tomato and potato had a folate content that had increased by double [26]. Due to the rise in demand for biofortified products, plant breeding on a genetic basis must be employed to boost output. Setting criteria is required to grow vegetables that will satisfy farmers' needs and the needs of the target population in terms of minerals and vitamins. It's critical to maintain vegetable output and production in the beginning so that farmers would adopt it and see a return on their investment. Farmers should be exposed to and educated about the worth of crops to increase their awareness of the need to cultivate biofortified veggies [68].

To speed up and reduce the time it takes to produce new crop cultivars, alternative methods like doubled haploidy breeding and marker-assisted selection can be added to the conventional breeding processes [59]. With an emphasis on horticultural crops like cassava, provitamin A has been developed. In the CIAT core sample, variations in the quantity of -carotene detected in cassava roots were examined (5500 genotypes). Cassava was found to have between 0.1 and 2.4 mg of -carotene per 100 g during breeding [7]. The biofortification breeding program for iodine and selenium is advised to be carried out concurrently with the primary objective for the thyroid and its smetabolism [35]. Some research improves agronomic practices in addition to breeding efforts to improve outcomes. It was discovered that after being fertilized with iodine and treated with diatomaceous earth, the Chinese cabbage (*Brassica chinensis* L.), spinach (*Spinacia oleracea* L.), and radish (*Raphanus sativus* L.) had significant iodine concentrations in their leaves [69]. In a different experiment, it was found that iodate ions had a less negative impact on the biomass production of spinach than iodide ions [74]. Radioactive iodine was employed by in a research to assess the iodine content and absorption in tomato fruit [31] and it was discovered that iodine was given to plants hydroponically, which resulted in increased iodine concentrations.

During the screening procedure, it is important to emphasize the rate and amount of micronutrient accumulation in the edible area [45]. After the high-yielding, high-vitamin, and nutrient lines are developed, they must be tested in a variety of habitats to demonstrate their ability to flourish in conditions specific to each location. Traditional plant breeding is a well-liked and inexpensive technique for developing resilient, biofortified plants. Plant breeding may be used to effectively and sustainably give minerals and vitamins to the target population. Nevertheless, polygene mostly regulates and has little impact on the absorption and accumulation of micronutrients in crops like vegetables. Hence, traditional biofortification breeding methods have had very limited success [40].

Genetic engineering approaches

Genetic engineering (GE) is often cited as a technique that will be essential for meeting future demands for food, feed, and energy. It was a record when the Flavr-Savr tomato was first made publicly available in 1996. Worldwide, biotechnology is being applied as a potent biofortification technique to address the significance of vitamin and mineral deficiency. With recent developments in genetic engineering tools and procedures, it is now feasible to add characteristics that traditional breeding cannot provide [53]. The latest advancements in plant breeding techniques, such as genome-wide association studies (GWAS) and marker-assisted breeding (MAB), are the most important and successful tools for biofortification. These recently developed methods assisted breeders in identifying the QTLs that increased levels of -carotene and -tocopherol by 3.22 and 5.76 times, respectively [2, 46].

While creating a crop to enhance a certain component, it is important to keep in mind the objective of employing genetic engineering to biofortify vegetables. Before a plant may absorb a micronutrient that is fixed in the soil, the micronutrient must first be made accessible to the plant. Many transporter mechanisms in plant cells let plants take up nutrients from the soil [64]. The use of genetics should improve the effectiveness of these mineral uptakes [50]. Redistributing micronutrients across the whole plant system is the second objective. The link between the source and drain will make it simpler to maintain the nutrients in the plant system. Although foliar treatment may promote the accumulation of micronutrients such as zinc in the shoot, zinc transport in the phloem precludes accumulation in fruit, seeds, and tubers [70]. The technique's last stage is employing a genetic tool to boost the biochemical process' effectiveness in edible tissues. Also, the antinutritional molecule, which eventually impairs the bioavailability of micronutrients, may be lessened by the biotechnological technique. Last but not least, overexpression of the gene that controls nutrient bioavailability may lead to an increase in the synthesis of nutritional enhancers [55]. Not to add, the new way causes the plant's edible component to produce more vitamins, whose use may lower the prevalence of malnutrition. Cassava and melons are examples of transgenic plants that outperform non-transgenic plants in terms of the bioaccessibility and bioavailability of carotene from plant matrixes [16, 19, 61]. Processing and heating are the main determinants of bioaccessibility and bioavailability. The bacterial approach was introduced to the potato, where the MEP pathway turns geranylgeranyl diphosphate into -carotene (provitamin A). Transgenic potatoes were found to have carotenoid and -carotene levels that were 20 and 3,600 times higher, respectively [14]. By inhibiting the process' rate-limiting enzyme, this method also enables the creation of transgenic plants. By silencing the ZEP gene, a transgenic potato was created that was biofortified with zeaxanthin and beta-carotene [56]. Zeaxanthin concentration in potato tubers was found to rise 130-fold under the transgenic line. Lycopene and beta-carotene levels in tomatoes may range from 5 to 15%, depending on the genotype and variety. Using the 35S::RNA polymerase, phytoene synthase and phytoene desaturase were overexpressed to create the transgenic method. Promoter for tp::crtI. There was a rise in cyclic carotenoids on the leaves of crtI tomatoes [14]. In cassava and potato, the *Chlamydomonas reinhardtii* gene *FEA1* is overexpressed, which significantly increases Fe buildup in edible tuber tissue [41]. Some of the examples in biofortified vegetable crops through genetic engineering are given in Table 3.

The transgenic strategy was used in various studies to increase Fe content. Using the rice glutelin-1 promoter, the transgenic plant's Fe content was enhanced and in comparison, to the control, the rise in Fe content was considerable [39]. One method for biofortifying plants is to overexpress the gene for Fe (III) reductases, which improves Fe absorption in non-graminaceous plants [9]. The bioavailability of micronutrients is decreased by antinutrients such as phytic acid and tannins, which prevent Fe, Zn, and Ca from being absorbed in the stomach of both humans and animals [68]. The phytate content in the edible part of wheat grains, which varies intra-specifically, is not much affected by the quantities of Fe and Zn. Iron and zinc cannot be absorbed because of phytic acid; thus, heating and processing are necessary to increase their bioavailability [30]. The bioavailability of minerals was said to be increased by inhibiting IP6 pathway enzymes. Moreover, increasing the quantity of phytase and phytate-degrading enzymes in the edible fraction may also reduce the amount of phytic acid, enhancing the bioavailability of micronutrients [22]. Iodine has a number of vital functions in the process of growth and development and is necessary for the hormones generated by the thyroid gland in humans [63]. The potato's Se content was raised by cautiously adding Se in tropical conditions. The concentrations of Se and Ca were also revealed to have risen [13].

Conclusion

A biofortification program might significantly enhance the quantity of vitamins and minerals in plants. This will assist in addressing the problem of malnutrition, sometimes known as "the hidden hunger" in underdeveloped nations. Increased output and productivity, abiotic stress tolerance, and biotic stress resistance are the main goals of many breeding initiatives. Yet, improving vegetable quality would aid less developed nations in saving money that might be utilized to treat diseases brought on by a lack of minerals and vitamins. Plant breeders, plant physiologists, biochemists, molecular biologists, and other nutrition specialists must work together to biofortify vegetables. Before being commercialized, the genetically engineered crop may require regulatory permission from many different organizations. More specifically, agricultural plants, including a range of vegetables, are being edited using CRISPR/Cas9 technology.

Future scope of study

Understanding the mechanisms of ion intake from the soil, redistribution within tissues, and plant homeostasis is necessary for improving micronutrient absorption by the plant. Recent advancements in genetics and genome editing technologies (TALENS, CRISPR/Cas9, etc.) may hasten the success of this biofortification endeavor.

Declaration

The authors declare no conflict of interest

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Table 1. Vegetable crops with various biofortified elements

Crop	Biofortified element	References
Cowpea	I and Zn	[34]
Green beans	I, P and Mg	[34]
Turnip	Se, Mg, P, Zn, Mn and Cu	[33]
Radish	Se	[72]
Carrot	I, Fe and Se	[60]
Broccoli	Zn, P, S, K, Fe, K, Cu, Mn	[36]
Cucumber	K and Ascorbic acid	[38]
Spinach	N, K	[11]
Lettuce	Fe, P, K, I, Zn, Se	[15]
Pepper	I	[32]
Tomato	Se	[54]
Amaranthus	Fe	[1]

Table 2. Biofortified varieties in vegetable crops developed through selection

Crop	Variety	Content-rich in	References
Potato	Kufri Neelkanth	Anthocyanin 100µg/100g	[20]
Sweet potato	Bhu Krishna and Bhu Sona	Anthocyanin (90mg/100g) and Carotene (14mg/100g)	[73]
Cauliflower	Pusa Beta Kesari 1	Carotene 8 to 10 ppm	[20]
Carrot	Ooty-1	Carotene (38 mg/100 g)	[73]

Table 3. Biofortified varieties in vegetable crops developed through genetic engineering

Crop	Gene	Content	References
Carrot	CAX1	Calcium	[73]
Sweet Potato	IBOR-INS	Lutein and Carotene	[47]
Lettuce	Ferritin	Iron	[58]
Potato	AmA1	Protein	[6]
Cauliflower	Or gene	Beta-Carotene	[28]
Cassava	PSY	Vitamin A	[58]

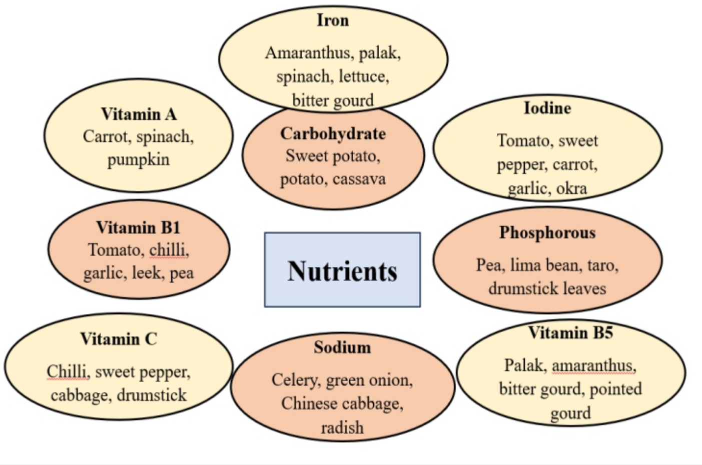

Figure 1. Nutrients in different vegetable crops

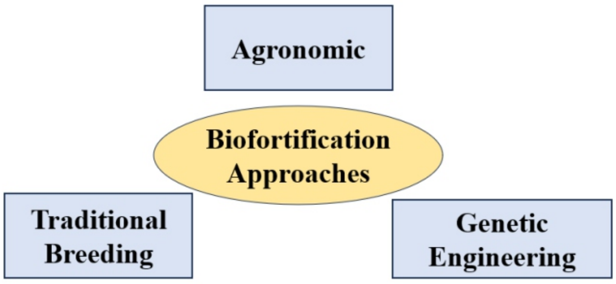

Figure 2. Different biofortification approaches

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