A Comprehensive Review on Biofortification in Vegetable Crops

Abstract

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Biofortification is an approach that combines biotechnology and fortification to improve the nutritional profile of staple crops, combating human malnutrition caused by essential vitamin and mineral deficiencies. It involves conventional breeding, biotechnology and agronomic strategies to increase the concentration of plant-derived nutrients and vitamins in edible parts during growth. While not as nutrient-dense as supplements or fortified foods, biofortified crops enhance daily micronutrient intake for all age groups. This sustainable, affordable approach is crucial for addressing micronutrient malnutrition, affecting around two billion people, particularly those with iron deficiency anaemia. Strategies include agronomic biofortification, involving micronutrient fertilizers and conventional breeding and hybridizing varieties for increased micronutrient levels. Genetic engineering enhances nutrient synthesis, while agronomic biofortification improves nutrient absorption and distribution. However, acceptance challenges and regulatory complexities persist. Future efforts should involve collaboration between plant breeders, molecular scientists and nutrition experts to enhance crop nutrition and receive increased funding from various organisations to combat global malnutrition effectively.

Keywords: Biofortification, Genetic engineering, Hidden hunger, Malnutrition, Vegetables

Introduction

Rising populations, insufficient food and nutrition, hunger, vitamin and micronutrient malnourishment, *etc*. are the main issues faced by the majority of the countries in the world . Vitamin-A deficiency (VAD), is more common in developing nations among women and children and causes over 600,000 deaths annually among children under five. About 60% of iron, 30% of zinc, 30% of iodine and 15% of selenium are among the micronutrients that the population is undernourished. Numerous physical and health issues in humans have been linked to the inadequate availability of these crucial vitamins and minerals. Conventional farming methods can improve the nutritional content of plant foods to some extent, but biofortification is the process of incorporating nutrients into food crops through conventional, agronomic and transgenic breeding techniques in order to address the long-term and sustainable effects of vitamin and nutrient deficiencies ??? (source). In most horticultural crops, including cowpea, pumpkin, banana, cassava, beans and orange fleshed sweet potatoes (OFSP), biofortification techniques have been used. There are already a number of conventional and transgenic cultivars available and more are being developed. Biofortification appears to be a viable technique in the fight against hidden hunger, as evidenced by the findings of efficacy and effectiveness studies and recent delivery successes (Source/s???).

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Biofortification is a combination of two terms namely bio originated from a *Greek* word which means life, and fortification a *Latin* word that means strengthening. Biofortification aims to enhance the nutritional content of crops, thereby mitigating the adverse impact of human malnutrition resulting from deficiencies in essential vitamins and minerals (Vandana *et al.,* 2022). It is the enhancement of micronutrient levels in staple crops (cereals and vegetables) through conventional breeding, biotechnology and agronomical tools. It enhances the nutritional content of crops by increasing the concentration of plant-derived nutrients and vitamins in the edible parts during plant growth and development (O'Hare, 2015).

Although biofortified foods are not as nutrient-dense as supplements or industrially fortified foods, they can enhance daily micronutrient intake for individuals of all age groups (Bouis *et al.,* 2011). Biofortification is either a cure for micronutrient deficiencies ~~nor~~ or a solution for all lacuna. It is one of the strategies to address micronutrient malnutrition in a sustainable, affordable and cost-effective manner. It has the potential to positively impact approximately two billion individuals suffering from iron deficiency anaemia, according to the World Health Organization (WHO) (McLean *et al.,* 2009).

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From an economic perspective, biofortification represents a single investment that provides a cost-efficient, enduring, and sustainable solution to combat pervasive malnutrition. Once biofortified varieties are established, they eliminate the need for ongoing expenses related to purchasing and incorporating fortifying agents during food processing. Additionally, the anticipated population growth in developing regions, coupled with evolving climate conditions, is expected to intensify the challenge of ensuring food security in the coming years (Bazuin *et al*.,2011). Consequently, prominent organizations like the World Health Organization and the CGIAR have prioritized the creation of nutritionally superior, high-yielding biofortified crops as a key objective (Bouis, 2000).

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Why biofortified crops are important?

- It increases the nutrient content of crops
- It is sustainable, affordable and lasting
- It helps people in isolated rural areas to improve their nutrition and fight against malnutrition

- It enhances the daily diets of people with enhanced vitamins and minerals (Vandana *et al.*, 2022)

Strategies for biofortification of vegetables

There are three ways to increase the nutritional value of vegetable crops through biofortification:

- **Agronomic Biofortification:** Applying fertilizers and other substances to enhance mainly micronutrient levels in plants along with the macronutrients.
- **Conventional plant breeding:** It involves hybridizing different crop varieties to create offspring with increased micronutrient levels.
- **Genetic engineering:** It involves incorporating genes from other organisms into crop plants to enhance the synthesis of nutrients and vitamins (Sources???).

I. Agronomic Biofortification

Agronomic biofortification is the process of application of micronutrient fertilizers to plants through soil application or by spraying. This helps enhance nutrient value in the edible part of the plant. This method is quick, efficient and simple but it has a short-term effect (Gomathi *et al.*, 2019). Zinc ($ZnSO_4$ sprayed on leaves), Iodine (iodide or iodate applied to soil) and Selenium (selenate form) are the best micronutrients for agronomic biofortification. Many observations find that mycorrhizal fungi improve the levels of Fe, Se, Zn and Cu in crops (Vijayreddy, 2024; Vijayreddy, 2023). Sulphur oxidising bacteria boost the sulphur content in onion. The foliar application of micronutrients to the leaves can enhance the absorption and distribution of nutrients in the food parts of plants more efficiently than soil application (Prasad *et al.*, 2015).

Limitations

Among all biofortification methods, agronomical biofortification is the simplest. However, agronomical biofortification efficacy is influenced by several factors, such as:

1. Mineral mobility and accumulation patterns in different plant species
2. Plant species diversity and their mineral demands
3. Soil properties and variations in different geographical locations (Ismail *et al.*,2007)

Biofortification of various nutrient contents in vegetable crops

1. Biofortification of Iron

Tomatoes are well suited for iodine biofortification initiatives because they can tolerate elevated iodine levels. Both their vegetative tissues and fruits can store iodine at concentrations that exceed human requirements. The amount of iodine found in the fruit of the plants treated with 5 mM iodide was more than sufficient to cover 150 µg of daily intake for humans. Using *Spirulina platensis* as a microbial inoculant can boost the iron content of Amaranthus plants compared to the control group. He also stated that *S. platensis* can improve the iron levels in the *Amaranthus gangeticus* plant as a biofortification agent (Kalpana *et al.,* 2014).

2. Biofortification of Zinc

The use of organic fertilizer Riverm helps the enrichment of Zinc (Zn) content in tomatoes, brinjal and sweet peppers compared to unfortified vegetables. Biofortified vegetables have 6.60–8.59% more zinc than the others (Yudicheva, 2014). Applying zinc through foliar and soil methods enhances the zinc content accumulation in broccoli during biofortification (Martin *et al.,* 2020).

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3. Biofortification of Selenium

Selenium (Se) biofortification in vegetable crops involves applying different concentrations of sodium selenite (Na_2SeO_3) through fertigation to enhance the selenium content in tomatoes. The application of 5 mg/l of sodium selenite, out of all the treatments, demonstrated the highest levels of selenium content in various plant parts and fruits (Rahim *et al.,* 2020). Carrots and broccoli were bio-fortified by applying a Se solution that was enhanced with Se content (Banuelos *et al.,* 2015)

II. Conventional plant breeding

Over the past forty years, traditional breeding has primarily concentrated on yield attributes and resistance breeding; this has resulted in a decrease in the nutritional status of the existing varieties due to a lack of emphasis on nutritional aspects (why not source??). Instances of the minerals Iron (Fe), Zinc (Zn), Copper (Cu) and Magnesium (Mg) have seen a decline in their mean concentration in the dry matter of several plant-based foods. Important vitamins, antioxidants and micronutrients have been fortified in conventional plant breeding as a result of recent advancements. Sufficient genetic diversity exists among cultivars in terms of β-carotene, functional carotenoids, Fe, Zn and other minerals (which nutrient for which crop must be indicated). This diversity enables the selection of nutritionally suitable breeding materials and is essential for enhancing the micronutrient content of staple foods through conventional breeding (Prasad *et al.,* 2015).

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Different methods of convectional breeding for biofortification of vegetable crops

1. Introduction

Plant introduction involves relocating plants from their original growth area to a new location where they are not cultivated. These plants can be either wild or entirely new crop varieties suited for the region. Notably, CIP-440127 from CIP in Peru, with a carotene content of 6.2–7.6 mg/100g and ST-14 from Japan, with a carotene content of 13.2–14.4 mg/100g, have been introduced for sweet potatoes(Yes, but where is the source of information. These are sweet potato varieties what about other vegetables??, very shallow information)~~.~~

2. Selection

A) Pure line selection

Three relatively distinct steps are typically involved in pure-line selection: (1) a large number of superior-appearing plants are selected from a genetically variable population; (2) progenies of the individual plant selections are grown and assessed by simple observation, often over a number of years and (3) when selection is no longer possible based solely on observation, extensive trials are conducted, involving meticulous measurements to ascertain whether the remaining selections are superior in yielding ability and other performance aspects. A new pure-line variety is then introduced if any offspring outperforms an already-existing variety (Upadhyay *et al.,* 2012). Table 1 depicts the different crop varieties that are biofortified using pure line selection.

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B) Mass selection

In mass selection, seeds are gathered from favourable individuals in a population (often a few dozen to a few hundred), and the resulting generation is seeded from a mixed seed stock. This technique, also known as phenotypic selection, is based on how each individual appears.

Mass selection has been employed extensively to enhance ancient land types (Garg *et al.*, 2018). Table 2 depicts the different crop varieties that are fortified using mass selection.

3. Mutation Breeding

Mutation breeding is that the deliberate induction and growth of mutant lines for crop improvement. Its most ordinarily utilized in asexually propagated crops and self-pollinated crops. Natural selection operates to cause about evolution of latest races and species through the variability created by natural mutations and amplified by subsequent recombination of genes during amphimixis Needs source!. Besides natural mutations that occur spontaneously caused by various sorts of radiations and cosmic rays received from the sun and also emitted by several radioactive elements, mutation also can be artificially induced by a variety of physical agents like gamma rays and X-rays and number of other sorts of chemical agents belonging to few specified groups referred to as chemical mutagens (sources??). And using an equivalent effectively through elaborate method of selection techniques in various generations for improvement of a specific crop species for desired objectives is named mutation breeding (Wilde *et al.*, 2012). Example-Mutation in peas leading to high iron content (Shallowely sourced!!)

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4. Hybridization

This process involves crossing two genetically distinct parents to create new crop varieties. The primary goal of hybridization is to produce diversity. Both cross-pollinated and self-pollinated crops can benefit from hybridization. The objective of hybridization is to combine beneficial genes from two or more distinct varieties in order to create purebred offspring that are superior to the parental types in many ways. Table 3 represents the biofortified crop varieties in vegetable crops produced by the hybridization method.

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Breeding in different crops for biofortification of nutrients

i. Breeding in Potato

Potato tubers have the highest number of antioxidants in human food. The different types of potatoes which have red and purple colours may provide more antioxidants to human health Sources!!!. That is why breeders try to create more of these kinds of potatoes. For the purpose of enhancing the quantity of iron and zinc in the diet of humans, potatoes can be bred to have a higher genetic diversity in micronutrients. The antioxidant and mineral composition of more than 1,000 different varieties of potatoes raised in the South America Andes were studied (copper, iron, manganese and zinc) source!!. The International Potato Centre (CIP) and Harvest Plus crossed disease-resistant tetraploid clones with high iron and zinc diploid Andean potatoes to create high iron and zinc potato breeding material. Rwanda and Ethiopia are the main countries where biofortified potato is targeted. The Potato Program of the National Institute for Agrarian Innovation (INIA) in Peru created INIA 321 Kawsay, a potato cultivar with high iron and zinc levels (Haynes *et al.,* 2012).

ii. Breeding in Cauliflower

When the genetic variation of zinc content in the *Brassica oleracea* gene pool—which includes cauliflower was tested, sufficient natural variation was discovered. Provitamin A (beta-carotene; 800–1,000 µg/100g) is abundant in the orange cauliflower variety Pusa Beta Kesari, which was developed in the Indian Agricultural Research Institute (IARI), New Delhi. Around the world, there are numerous other varieties of cauliflower that are coloured differently. For example, orange and purple cauliflowers contain beta-carotene and anthocyanin, respectively. Purple Graffiti and Orange Cheddar are two coloured cauliflower varieties developed by Cornell University in the United States (Broadley *et al.,* 2010).

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iii. Breeding in Cassava

A common root vegetable in developing and under-developing nations is cassava, which is particularly popular in Africa, Latin America and the Caribbean. Africa's cassava crop now contains more provitamin A (beta-carotene) thanks to a partnership between Harvest Plus and the International Institute of Tropical Agriculture in Nigeria (TMS 01/1368—UMUCASS 36, TMS 01/1412—UMUCASS 37 and 2014; TMS 01/1371—UMUCASS 38 and NR 07/0220—UMUCASS 44, TMS 07/0593—UMUCASS 45 and TMS 07/539—UMUCASS 46), as well as in the Democratic Republic of the Congo (DRC) [Kindisa (TMS 2001/1661)], six new types of cassava with increased vitamin A have been developed. Several gene types found in cassava also influence the number of proteins, minerals (such as iron and zinc) and carotene it contains (Chavez *et al.,* 2005).

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iv. Breeding in Tomato

Tomato is a rich source of vitamins A and C and a significant crop. Tomato breeding has used the genetic diversity of wild tomatoes to search for desirable characteristics (Rick and chetelat, 1995). A conventional breeding method has produced Sun Black, an anthocyanin enriched tomato with a dark purple skin from high anthocyanin levels in the peel (Mazzucato *et al*., 2008). Another variety, Black Galaxy, was created by the same method in Israel. Table 4 shows the various varieties that have been bred through conventional breeding to develop biofortified varieties in various nations.

Conventional breeding limitations

Conventional plant breeding methods also have some limitations

1. Low genetic diversity for the micronutrients in the plant gene pool and the long duration required to produce cultivars with the wanted trait(s).
2. It would be unfeasible to breed for a certain trait by conventional methods, the time and work needed may be impractical.

3. Those with recessive genes are not easy to breed conventionally.

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III. Genetic engineering

Around the world, biotechnology is being utilized as an efficient biofortification method to address the severity of vitamin and mineral deficiencies. Recent advancements in genetic engineering tools and methods make it possible to incorporate traits that conventional breeding is unable to produce(sources!!. The direct manipulation of an organism's genes through the use of biotechnology is known as genetic engineering or genetic modification (Vijayreddy, 2023; Dutta *et al.,* 2023). Genetic engineering enables the creation of elite cultivars by transferring desirable traits from one organism to another, significantly enhancing the value of these cultivars (Athar *et al.,* 2020). Table 5 enlisted the usage of various genes to develop biofortified vegetable crops.

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Biofortification can enhance the levels and availability of micronutrients in edible crops. It also presents special chances to enhance nutritional quality and provide additional health advantages. Numerous vegetable crops have undergone genetic modification to enhance traits such as nutritional quality, flavour improvement, bitterness reduction and increased content of anthocyanin, carotene, calcium, protein, lutein and iron (Bouis and Saltzman, 2017).

Limitations in biotechnological biofortification method

1. **Genetic Variation**: Transgenic crops address the constraint of limited genetic diversity found in conventional breeding.
2. **Acceptance Challenges**: However, their acceptance among the general population remains low.

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3. **Regulatory Processes**: Different countries have varying regulatory procedures for approving and commercializing transgenic crops, which can be costly and time-consuming (Inaba *et al*., 2004)

Future thrust

Biofortification is the process of increasing the nutritional value of crops by using various methods, such as plant breeding, agronomic practices and genetic engineering. Many plant breeding programs aim to enhance productivity, stress tolerance and taste, but nutritional quality is also a recent goal. To achieve these goals, plant breeders need to work with nutrition scientists. However, some biofortification programs are not feasible because of the low genetic diversity for micronutrients in the plant material. In these cases, genetic engineering is required and collaboration with molecular biologists is necessary. The main challenge for the commercialization of Genetically Modified Crops (GM Crops) is the costly and lengthy regulatory approval process (Kumar *et al.*, 2023). Biofortification is a potential agricultural solution for improving the nutrition of undernourished populations throughout the world. Therefore, biofortification programs should receive more funding from the various global organisations.

Conclusion

Nutritional security is a key research area for developing countries after food security. Many people in the developing world face hidden hunger due to lack of micronutrients and vitamins. Agricultural scientists can modify the physiology of crops to increase the nutritional value of vegetables and cereals. Plant breeders, molecular scientists and genetic engineers have many opportunities to enhance the micronutrient and vitamin content of staple food crops and vegetables for developing countries. The new varieties that are rich in micronutrients and vitamins should be widely adopted by farmers without affecting their yield and quality. There is a lot of genetic variation in vegetables that can be used to select for specific traits. To increase the micronutrient content of plants, the mechanisms of ion uptake from soil, redistribution within tissues and homeostasis in the plant should be understood. Both conventional breeding and genetic engineering require specific traits to be introduced. The latest developments in genetics enable the reduction of anti-nutrients such as phytic acid or tannins to increase the micronutrient content. Genome editing tools such as ZFN, TALENS, CRISPR-Cas9, *etc.* can alter or remove unwanted genes and can be used for the biofortification of vegetables.

Please the objective of the review is not well stated and hence its conclusion is also became too general rather than specific.

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Tables

Table 1: Different Biofortified varieties of vegetable crops developed through Pure line selection

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Crop	Variety	Content	References
Cauliflower	Pusa Beta Kesari	β-carotene (8.0-10.0 ppm)	Garg *et al.,* 2018
Sweet potato	Bhu Sona	β-carotene (14.0 mg/100g)	Yadava *et al.,* 2020
	Bhu Krishna	Anthocyanin (90.0 mg/100g)	

Source!!

Table 2: Different biofortified varieties of vegetable crops developed through mass selection

Crop	Variety	Content-rich in	References
Carrot	Madhuvan Gajar	β-carotene content (277.75 mg/kg) and iron content (276.7 mg/kg)	Yadava *et al.,* 2020
	Ooty-1	Carotene (38 mg/100 g)	Yadava *et al.,* 2020
Cowpea	Pant Lobia-1	82 ppm Fe and 40 ppm Zn	Golob *et al.,* 2020
	Pant Lobia-2	100 ppm Fe and 37 ppm Zn	Golob *et al.,* 2020
Potato	Kufri Neelkanth	Anthocyanin (100µg/100g)	Garg *et al.,* 2018
Pumpkin	Arka Chandhan	Carotene (3333 IU)	Carvalho *et al.,* 2014
Radish	Pusa Gulabi	Anthocyanin	Yadava *et al.,* 2020

Source!

Table 3: Different Biofortified varieties of vegetable crops developed through Hybridization

Crops	Variety	Content-rich in	References
Watermelon	Arka Jyoti, Durgapur Lal	Carotene	Gomathi *et al.,* 2019
Brinjal	Punjab Sadabhar	Anthocyanin	Gomathi *et al.,* 2019
Okra	Kashi Lalima	Anthocyanin (3mg/100g)	Rahim *et al.,* 2020
Bitter gourd	Pusa hybrid 4	Iron (18.28 mg/100g)	Golob *et al.,* 2020

Carrot	Kashi Krishna	Anthocyanin	Yadava *et al.,* 2020
Tomato	Pusa Rohini	Vitamin C (3.12mg/100g)	Carlo *et al.,* 2020

Source!

Table 4: Different biofortified vegetable crops developed through conventional breeding in different countries

Crop	**Variety**	**Content-rich in**	**Released in**	**References**
Tomato	Sun Black	Anthocyanin	Israel	Mazzucato *et al*., 2008
	Black Galaxy			Mazzucato *et al*., 2008)
Potato	INIA321 Kawsay	Rich in iron and Zinc	Peru	Haynes *et al.,* 2012
Sweet potato	Ejumula, Kakamega, Vita kabode, Naspot 120 and Naspot 130	Vitamin -A	Uganda	HarvestPlus, International Potato Centre (CIP)
	Twatasha, Kokota and Chiwoko	Vitamin-A	Zambia	HarvestPlus, International Potato Centre (CIP)
Cassava	UMUCASS 36 (TMS 01/1368), UMUCASS 37 (TMS 01/1412), UMUCASS 38 (TMS 01/1371), UMUCASS 44 (NR	Vitamin-A	Nigeria	Chavez *et al.,* 2005

	07/0220), UMUCASS 45 (TMS 07/0593), UMUCASS 46 (TMS 07/0539), TMS 2001/1661			

Source!

Table 5: Biofortified vegetable crops developed through transgenic engineering

Crop	**Gene**	**Content**	**References**
Potato	AmA1	Protein	Chakraborty *et al.,* 2010
	Strawberry d-galacturonic acid reductase (GaIUR) gene	Vitamin C	Hemavathi *et al,* 2009
	PSY, phytoene desaturase and lycopene Beta cyclase	Vitamin A	Diretto *et al*., 2004
	beta-carotene hydroxylase gene (bch)	β- carotene	Van Eck *et al.,* 2007
	Perilla [PrLeg polypeptide and cystathionine γ-synthase (CgS) genes	Methionine	Goo *et al*., 2013; Di *et al*., 2003
Tomato	Tomato pds-beta Lyc	β- carotene	Rosai *et al*, 2000
	CaMV35S: crtl	β- carotene	Romer *et al*., 2000
Cauliflower	Or gene	β- carotene	Yunhua Ding and Yuancai Jain,2007

Lettuce	Soya bean Ferritin gene	Iron	Sharma et al., 2017
Sweet Potato	IBOR-INS Lutein	Lutein and Carotene	Park *et al.,* 2015
	IbMYB1	Anthocyanin	Park *et al.,* 2015
Cassava	PSY	Vitamin A	Welsch *et al*., 2010
	nptII, crtB and DXS	β- carotene	Telenech *et al.,* 2015
Carrot	CAXI	Calcium	Yadava *et al.,* 2020

Source!