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Biofortification of vegetable crops – A Review

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Abstract
Malnutrition is responsible for severe social and health concerns and therefore, intensifies acute anxiety throughout the world. Nutrition is the strategic element in several stratagems designed to ease the burden of diseases on a global level. The green revolution satisfied the necessity for greater yield but at the expense of quality. Today, poor people are primarily suffering from micronutrient malnutrition as they cannot afford dietary supplementation due to poverty. Hence, the production of bio fortified food crops is necessary to resolve the problem of micronutrient deficiency on a sustainable basis. Biofortification of commonly consumed food crops offers the simplest solution to complex nutritional disorders. Biofortification is the process of adding nutrients to food crops, which provides a sustainable and long-term means of delivering more micronutrients. Currently, agronomic, conventional and genetic engineering are three common approaches. Agronomic biofortification can provide temporary micronutrient increases through fertilizers. In conventional plant breeding, parent lines with high vitamin or mineral levels can be crossed over several generations to produce plants that have the desired nutrients. Genetic engineering techniques are utilised to produce new cultivars through the transfer of desirable characters from one organism to another to develop elite cultivars, thereby improving their value.

Keywords: Vegetables, biofortification, breeding, genetic engineering

Introduction
Malnutrition is the condition that develops when the body is deprived of vitamins, minerals and other nutrients it needs to maintain healthy tissues and organ function. Malnutrition is responsible for severe social and health concerns and therefore, intensifies acute anxiety throughout the world. Nutrition is the strategic element in several stratagems designed to ease the burden of diseases on a global level. The green revolution satisfied the necessity for greater yield but at the expense of quality. Today, poor people are primarily suffering from micronutrient malnutrition as they cannot afford dietary supplementation due to poverty.

Table 1: Deficiency of nutrients causes many diseases like

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

Nutrients can be enriched by 1) fortification, that is, by adding vitamins and minerals to commonly consumed foods during processing to increase their nutritional value 2) Supplementation is the use of vitamin and mineral supplements 3) Biofortification outperforms other methods in which nutrients are added to a crop or plant (Gomathi *et al.*, 2019) [32].

Biofortification is derived from two words, *i.e.*, bio is from a Greek word meaning life and fortificare is derived from a Latin word meaning to make strong. It refers to the nutrient enrichment of crops to address the negative economic and health consequences of vitamin and mineral deficiencies in humans (Vandana *et al.*, 2022) [29].

Importance of Biofortification

Biofortification provides a comparatively sustainable, cost effective and long term means of delivering nutrients to people in relatively remote rural areas to overcome malnutrition and increase the nutritional quality of their daily diets (Vandana *et al.*, 2022) [29]. According to a World Health Organization (WHO) estimation, biofortification could help cure two billion people suffering from nutrient deficiency. Biofortification supplements to provide micronutrients to the most vulnerable populations at a lower cost over time.

Methods of Biofortification

1. Agronomic Biofortification.
2. Conventional Breeding.
3. Genetic Engineering.

Agronomic Biofortification

Agronomic biofortification is the application of micronutrient - containing mineral fertiliser to the soil and/or plant leaves. This helps to increase the micronutrient content of the edible part of food crops. It is a short-term, effective and convenient method (Gomathi *et al.*, 2019) [32]. Prasad *et al.* (2015) [20] opined that foliar application of fertiliser with micronutrients often stimulates more nutrient uptake and efficient allocation in the edible plant parts than soil fertilization. (Saltzman *et al.*, 2017) [24] Stated that the combination of soil and foliar application is often the most effective method. Soilless cultivation is another method with precise control of plant nutrition that helps to enhance the concentration of valuable elements in the plant tissues (Montesano *et al.*, 2016) [33]. Martin *et al.* (2020) [17] conducted a study on soil and foliar application of zinc to biofortify broccoli. The result showed that combined soil and foliar application of zinc sulphate recorded more accumulation of zinc in broccoli. Rahim *et al.* (2020) [20, 21] studied the agronomic biofortification of tomatoes by the application of different concentrations of sodium selenite (Na_2SeO_3) through fustigation to increase the concentration of selenium in tomato plants and fruits. Among different treatments, application of 5 mg/L of sodium selenite showed the highest agronomic variables and selenium content in different parts of the plant and fruits. Broccoli and carrots were bio-fortified by foliar application of a solution of Se that was enriched with Se content (Banuelos *et al.*, 2015) [3].

Table 2: Nutritional trait improvements through agronomical biofortification

Crop	Targeted micro-nutrient	References
Legumes		
Cowpea	I and Zn	Lopez *et al.*, 2020 [15]
Green beans	I, P and Mg	Lopez *et al.*, 2020 [15]
Root crops		
Turnip	Se, Mg, P, Zn, Mn and Cu	Li and Yang, 2018 [14]
Radish	Se	Woch and Hawrylak-Nowak, 2019 [30]
Carrot	I, Fe and Se	Smolen *et al.*, 2019 [26]
Cole crops		
Broccoli	Zn, P, S, K, Fe, K, Cu, Mn	Martin *et al.*, 2021 [18]
Cucurbit crops		
Cucumber	K and Ascorbic acid	Montoya *et al.*, 2013 [34], Anjanappa *et al.*, 2012 [1]
Pumpkin	Se and I	Golob *et al.*, 2020 [35]
Leafy Vegetables		
Spinach	N, K	Darshan *et al.*, 2019 [8]
Lettuce	Fe, P, K, I, Zn, Se	Dobosy *et al.*, 2020 [9]
Solanaceous crops		
Pepper	I	Li *et al.*, 2017 [13]
Potato	I	Dobosy *et al.*, 2020 [9]
Tomato	Se	Rahim *et al.*, 2020 [20, 21]

Biofortification through Conventional Breeding

Conventional breeding is the science of changing the traits of plants in order to produce desired characteristics. The goals of plant breeding are to produce crop varieties that boast unique and superior traits. For the previous four decades, conventional breeding methods predominately focused on enhancing yield traits and developing resistant cultivars. This unidirectional approach ultimately results in reduced nutritional value in existing varieties. (Roriz *et al.*, 2020) [23]. the conventional breeding approach is one of the easiest and most convenient strategies to enhance the phyto-nutritional traits of vegetable crops. There is a need to identify the nutrient rich cultivars within the existing germplasm so that targeted nutrients can be incorporated into the crop to enhance the nutritional status (Gomathi *et al.*, 2017) [11]. Conventionally developed Biofortified foods are cost-effective and have wider acceptance among consumers than transgenic plants. Bouis and Saltzman (2017) [4] opined that the conventional approach is a more feasible means of biofortification of crops than the agronomic approach as they are not dependent on supplementation through synthetic fertilisers.

Different methods of Conventional breeding

1. Introduction: It is the process of introducing plants in the new locality from their own growing locality, which may involve wild or totally new varieties of crops for the area. In Sweet potato, CIP-440127 is introduced from CIP, Peru where carotene content is 6.2-7.6 mg/100g and ST-14 from Japan whose carotene content is 13.2-14.4 mg/100g (Tengali *et al.*, 2021) [27].

2. Selection

A) Pure line Selection: A large number of plants are selected. The selected plants are harvested individually. Selected individual plants are grown in individual rows and evaluated and the best progeny are selected (Upadhyay *et al.*, 2012) [28].

B) Mass selection: In this method, a large number of plants with similar phenotypes are chosen, and their seeds are bulked and used to grow the next generation (Garg *et al.*, 2018) [10].

Table 3: Biofortified varieties developed through selection

Crop	Variety	Content rich in	References
Potato	Kufri Neelkanth	Anthocyanin 100µg/100g	Garg *et al.*, 2018 [10]
Carrot	Ooty-1	Carotene (38 mg/100 g)	Yadava *et al.*, 2020 [31]
Pumpkin	Arka Chandan	Carotene (3333 IU)	Carvalho *et al.*, 2014
Cauliflower	Pusa Beta Kesari 1	Carotene 8 to 10 ppm	Garg *et al.*, 2018 [10]
Sweet potato	Bhu Krishna and Bhu Sona	Anthocyanin (90mg/100g) and Carotene (14mg/100g)	Yadava *et al.*, 2020 [31]
Cowpea	Pant Lobia-1	82 ppm Fe and 40 ppm Zn	Golob *et al.*, 2020 [36]
	Pant Lobia-2	100 ppm Fe and 37 ppm Zn	Golob *et al.*, 2020 [36]

3. Mutation breeding: It is referred to as "variation breeding." It is the process of exposing seeds to chemicals, radiation, or enzymes in order to generate mutants with desirable traits to be bred with other cultivars. Plants created using mutagenesis are sometimes called mutagenic plants.

4. Hybridization: It is the method of producing new crop varieties by crossing two genetically different parents. The main purpose of hybridization is to create variation. Hybridization is applicable to both self-pollinated and cross-pollinated crops.

Table 4: Biofortified varieties developed through hybridization

Crop	Variety	Content rich	References
Water melon	Arka Jyoti, Durgapur Lal	Carotene	Gomathi *et al.*, 2019 [37]
Brinjal	Punjab Sadabhar	Anthocyanin	Gomathi *et al.*, 2019 [37]
Okra	Khasi Lalima	Anthocyanin (3 mg/100g)	Rahim *et al.*, 2020 [20, 21]
Bitter gourd	Pusa Hybrid 4	Iron 18.28 mg/100g	Golob *et al.*, 2020 [36]
Carrot	Kashi Arun	Lycopene (7.5 mg/100g)	Yadava *et al.*, 2020 [31]
Radish	Khasi Lohith	Anthocyanin: (39.9 µg/100g)	Yadava *et al.*, 2020 [31]
Tomato	Pusa Rohini	Vitamin C (31.2 mg/100g)	Carlo *et al.*, 2020

Biofortification through Genetic Engineering

Genetic engineering, also called genetic modification, is the direct manipulation of an organism's genes using biotechnology. Genetic engineering enables to produce new cultivars through the transfer of desirable characters from one organism to another to develop elite cultivars, thereby improving their value considerably (Athar *et al.*, 2020) [2]. It offers the potential to increase the concentration and bioavailability of micronutrients in edible crops. It further offers unique opportunities for improving nutritional quality and bringing other health benefits. Many vegetable crops have been genetically modified to improve traits such as nutritional status or better flavor and to reduce bitterness, slow ripening, higher nutritional status, seedless fruit, increased sweetness and anti-nutritional factors (Bouis and Saltzman, 2017) [4].

Agrobacterium-mediated plant transformation process

The *Agrobacterium*-mediated transformation process involves a number of steps:

1. Isolation of the genes of interest from the source organism.
2. Development of a functional transgenic construct including the gene of interest; promoters to drive expression; codon modification, if needed to increase successful protein production; and marker genes to facilitate tracking of the introduced genes in the host plant
3. Insertion of the transgene into the Ti-plasmid.
4. Introduction of the T-DNA-containing-plasmid into *Agrobacterium.*
5. Mixture of the transformed *Agrobacterium* with plant cells to allow transfer of T-DNA into plant chromosome.
6. Regeneration of the transformed cells into genetically modified (GM) plants.
7. Testing for trait performance or transgene expression at lab, greenhouse and field level.

Table 5: Biofortified varieties developed through genetic engineering

Crop	Gene	Content	References
Tomato	*pGAntho*	Anthocyanin	Manamohan *et al.*, 2013 [16]
Potato	*AmA1*	Protein	Chakraborty *et al.*, 2010 [7]
Lettuce	*Ferritin*	Iron	Sharma *et al.*, 2017 [25]
Cauliflower	*Or gene*	Beta-Carotene	Kalia *et al.*, 2016 [12]
Sweet Potato	*IBOR-INS*	Lutein and Carotene	Park *et al.*, 2015 [19]
Cassava	*PSY*	Vitamin A	Sharma *et al.*, 2017 [25]
Carrot	*CAX1*	Calcium	Yadava *et al.*, 2020 [31]

Conclusion

In the current situation, hunger and malnutrition pose a major challenge, which can be overcome through nutrient-rich Biofortified vegetables with long-term benefits. It is both environmentally beneficial and economically viable. Many health problems can be prevented and controlled by increasing awareness about the benefits of many vegetables. Agronomic biofortification, conventional breeding methodologies and transgenic techniques are the most feasible means to improve the nutritional quality of vegetable crops. Conventional breeding is one of the most acceptable approaches because the improved cultivars could be utilised in the long term and do not have any health issues like agronomic and transgenic approaches.

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