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REVIEW

Biofortification of vegetable crops for vitamins, mineral and other quality traits

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ABSTRACT
Micronutrient 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. Henceforth, the production of biofortified 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. This review highlights different biofortification approaches that are engaged to offset numerous nutrient deficiencies along with new advancements to be undertaken. The impacts of various internal and external attributes have been discussed for SWOT analysis for scaling up the biofortification initiatives. Additional efforts to revise the prevailing genomes by employing molecular techniques can open new pathways in the research field of biofortification.

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Introduction

Biofortification is expressed as the process of nutrient enrichment or enhancing nutritional value to the crops that helps to reduce the deficiencies of vitamins and minerals (Prasad et al., 2015). This can be achieved using innovative interventions known as ‘nutrition-sensitive-agriculture’ or the ‘complementary-rural-targeted micronutrient program approach’ (Ansari & Thapa, 2019). Biofortification could be achieved by employing different approaches such as agronomic, conventional, or biotechnological. Biofortification as it emphases on enhancing the nutritional status of the cop naturally and differs from fortification where nutrient supplementation of the food items is done (Malik & Maqbool, 2020). Current scenario revealed that 800 million peoples all around the world are malnourished and 98% of which belong to developing countries (Sinha et al., 2019). Besides, about two billion global population have been experiencing hidden hunger (Gillespie et al., 2016). So, to overcome these situations, biofortification emerges as a socioeconomically feasible way to improvise the nutritional status of crops (Malik & Maqbool, 2020). The nutritional status of the crops depends on various aspects that may include crop species, variety, growing conditions and production strategies (Ceccanti, Landi, Benvenuti, Pardossi, & Guidi, 2018).

Vegetables signify one of the vital sources of nutrients in the human diet and are referred as ‘protective foods’ because of their ability to shield the body against numerous ailments. Minerals and vitamins constitute an integral component of human growth and development (Lal et al., 2020). The vitamins such as A, C, E, and beta-carotene, are effective antioxidants that also help in improving health by employing other mechanisms (Parulekar, Haldankar, Dalvi, & Bhattacharyya, 2019). The crops like amaranths, ash gourds, cabbage, curry leaf, fenugreek, palak, bitter gourd, spinach, lettuce, okra, onion, radish, and pea contain a fair amount of Ca, Fe, I, K, P & Na (Gomathi, Vethamoni, & Gopinath, 2017; Singh, 2020; Singh, Selvakumar, Mangal, & Kalia, 2020). Plant bio-fortification provides an innovative tool for increasing the bioavailability of micronutrients and improving the nutritional status of food crops. This approach could be adopted for biofortification by increasing the nutrient content in the edible portion of crop plants or by lowering the anti-dietary factors. Several barriers prevent the accumulation of trace elements in plants. One of the main obstacles to biofortification is the lack of knowledge about the available concentration of micronutrients in the seeds and the uncertainty about genes and pathways that alter targets (Waters and Sankaran, 2011). limate change has resulted in a significant reduction in nutrients accumulation such as nitrogen, phosphorus, potassium and iron in plants (Milius, 2017). Several barriers needed

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to be overcome for the enhanced accumulation of more micronutrients in the edible portion of plants. These might be controlled by homoeostasis, which has a crucial role in regulating the metal absorption, translocation, and redistribution in the plant body (Kulcheski, Correa, Gomes, de Lima, & Margis, 2015). The rhizosphere creates one of the barriers since soil is the key supplier of micronutrients to plants, where their bioavailability has been influenced by various soils and environmental factors (Rawat, Saxena, & Sanwal, 2019). Plant roots uptake nutrients from the ground and transport them to aerial plant parts. Before entering the plants, these nutrients have to pass through the concentric layers of the epidermis, root cortex, and endodermis. Differentiation of endodermis and its developmental plasticity function as barriers for nutrient translocation in plants (Barberon, 2017). Absorption mechanisms (ion channels and transporters) present in the plasma membrane of root cells also act as a barrier for the movement of nutrients to plants. If they are not active and specific, they obstruct the entry of micronutrients into the apoplasm of root cells, resulting in reduction in their translocation into the plant (Li et al., 2017). With advancements in the agricultural sector, new advanced modern techniques are involved in a series of crop biofortification. These recent era approaches include nano-technology, plant growth-promoting rhizobacteria-based biofortification, marker assisted selection, genetic engineering etc. These all aspects have been reviewed to gather knowledge for further implementation in the future.

Biofortification through agronomic approaches

Agronomic biofortification is one of the most frequent methods utilised for biofortification. In this approach, fertilisers are applied to enhance the micronutrient concentration in the edible portion of the plant (Prasad et al., 2015) through direct absorption of nutrients from the soil into the plant (Saltzman et al., 2017). The degree of success in this approach is proportional to the mobility of mineral elements either in soil or plants (Montesano et al., 2016). In addition to this, agronomic-biofortification offers temporary proliferation in micronutrients through fertilisers application (Saltzman et al., 2017). The soilless cultivation system is another method with precise control of plant nutrition that help to enhance the concentration of valuable elements in the plant tissues (Montesano et al., 2016). Legumes are known for containing a fair amount of boron, yet this element is not a vital part of the human nutrition diet (Hunt, 2012). Different reports revealed that boron could impose positive effects particularly on longevity, when consumed with 1.0–3.0 mg/day (Nielsen, 2018). In purslane, the amount of boron increased in edible portions about 1.8–10.7 fold without compromising crop performance when it was provided at 3 and 6 mg per litre in nutrient solution using a floating hydroponic system (Imperio et al., 2020). For nutrient biofortification in the cucurbits, soil application of nutrients can be the most convenient and cost-effective agronomical approach (Garg, Sharma, Sharma, Kapoor, & Arora, 2018) and numerous research studies have confirmed the increased status of targeted nutrients. In contrary, Preciado-Rangel et al. (2018) confirmed the positive response of the soil application of potassium at the rate of 11 mM in *Cucumis melo*, that augmented the antioxidant activity and phenol content. Meanwhile, Abd-Alkarim, Bayoumi, Metwally, and Rakha (2017) reported that ascorbic acid content was improved by the foliar application of silicon (100 to 200 mg L-1). Agronomic biofortification is a short-term effective and convenient method and various reports enhancing specific micro-nutrients are summarised in Table 1.

Biofortification through microbes

Plant growth-promoting rhizobacteria is an efficient method for improving the nutritional quality of leguminous crops. Legume crops can efficiently fix the atmospheric nitrogen through a symbiotic process that help to improvise the nutritional value and yield of the crop (Roriz, Carvalho, Castro, & Vasconcelos, 2020). Arbuscular mycorrhizal fungi and root endophytic fungi were reported to enhance the selenium content in vegetable crops and thereby, contribute to Se biofortification (Ye et al., 2020). For example, garlic biofortified with *Glomus irtraradices* increased the selenium content up to 10-fold (Larsen et al., 2006). Numerous successful examples of biofortification of vegetable crops through microbes have been illustrated in Table 2. Application of Zn solubilising bacteria and its combinations abundantly increased the zinc bioavailability in tomato up to 2.06 mg to 2.87 mg per 100 g (Karnwal, 2021a). Meanwhile, okra seed treatment with combined application of *Pseudomonas sp.* + *P. stutzeri* improved the zinc content concentration by 2.85 mg/100 g (Karnwal, 2021b).

Plant growth-promoting rhizobacteria have also been shown to increase the micronutrient content in plants by augmenting their supply (Bhardwaj, Ansari, Sahoo, & Tuteja, 2014; Sahoo, Bhardwaj, & Tuteja, 2013). Excess use of inorganic fertilisers which may lead to harmful environmental hazards is the foremost concern (Jewell et al., 2020; Schier et al., 2019). However, these fertilisers are costly and need more manpower, particularly for small land-holding

Table 1. Nutritional trait improvements through agronomical biofortification.

Crop	Targeted micro-nutrient/ other traits	References
Legumes		
Cowpea	I	Ojok et al., 2019
Cowpea	Zn	López-Morales et al., 2020
Green beans	I, P & Mg	Dobosy et al., 2020
Root crops		
Turnip	Se, Mg, P, Zn, Mn & Cu	Li, Li, & Yang, 2018
Radish	Se	Woch & Hawrylak-Nowak, 2019
Carrot	Fe & Se	Smolen et al., 2019
Carrot	I	Dobosy et al., 2020
Carrot	I &Se	Skoczylas, Tabaszewska, Smolen, Słupski, & Baranski, 2020
Cole crops		
Brassicaceae (micro-greens)	Zn & Fe	Gioia, Petropoulos, Ozores-Hampton, Morgan, & Rosskopf, 2019
Mustard sprout	Se	Woch & Hawrylak-Nowak, 2019
Broccoli	Zn, P, S, K, Fe, K, Cu, Mn	Rivera-Martin, Broadley, & Poblaciones, 2020
Cabbage	Se	Liao et al., 2021
Broccoli	N & Zn	Rivera-Martin, Reynolds-Marzal, Martin, Velazquez, & Poblaciones, 2021
Cucurbit crops		
Cucumber	K	Montoya, Ortega, Navarro, & Lorenzo, 2013
Pumpkin	Se & I	Golob et al., 2020
Leafy Vegetables		
Alfalfa	Se	Woch & Hawrylak-Nowak, 2019
Lettuce	Fe, P, K, I, Zn, Se	Giordano et al., 2019; Smolen et al., 2019 Dobosy et al., 2020, Almeida et al., 2020
Sweet Basil & Lettuce	I	Puccinelli, Landi, Maggini, Pardossi, & Incrocci, 2021
Curly Endive	I	Sabatino et al., 2021
Leafy greens	I	Ligowe et al., 2021
Solanaceous crops		
Pepper	I	Li et al., 2017
Potato	I	Smolen, Smolen, Rozek, Sady, & Strzetelski, 2020
Tomato	Se	Rahim, Rocio, Adalberto, Rosaura, & Maginot, 2020
Tomato	Se	Gaucin-Delgado et al., 2020
Cherry tomato	Se	Sabatino et al., 2021

farmers (Coyne et al., 2020). Besides, amount of synthetic fertilisers and time of application are major concerns in the case of soilless cultivation that need to be rectified for effective biofortification (Rouphael & Kyriacou, 2018). Additionally, the effectiveness of applied fertilisers may depend on soil status, crop phenotype, and genotype (Izydorczyk et al., 2021). However, diverse genotypes may show differences in their mechanism of nutrient uptake, accumulation, and translocation (Rosa, Diaz, Hansel, Sebastian, & Adee, 2019). For example, the application of iron fertilisers in calcareous soil, which has a high pH level that lowers the mobility rate of iron ions and converts the iron into unavailable forms (Ramzani, Khalid, Naveed, Irum, & Kausar, 2016). This is not a major limitation in soilless cultivation of crops, because of their cultivation under controlled environmental conditions (Barbosa et al., 2015).

Table 2. Biofortification of vegetables through use of microbes.

Crop	BMO/ PGPR types	Targeted Nutrients	Reference
A. sativum	Arbuscular mycorrhizal, Root endophytic fungi, PGPR	Se	Golubkina et al., 2019
Asparagus	Arbuscular mycorrhizal fungi	Se	Conversa et al., 2019
Tomato	Zinc solubilising bacteria	Zn	Karnwal, 2021a
Okra	Zinc solubilising bacteria	Zn	Karnwal, 2021b
Potato	Fe solubilising bacteria	Fe	Mushtaq, Asghar, Zahir, & Maqsood, 2021

Biofortification using nanotechnology

Nanotechnology offers several types of scientific applications and advancements in agricultural sectors (Elemike, Uzoh, Onwudiwe, & Babalola, 2019). Applied fertilisers impose a powerful impact on the bio-availability of the micronutrients, yield, and nutrient use efficiency of the plants (Thakur, Thakur, & Kumar, 2018). Apart from this, the micronutrient efficiency of commercial fertilisers ranged from 2.5–5% approximately. This efficiency of fertilisers is secure due to their properties such as low mobility rate within plant parts, less rigorous dispersion of micronutrients into the leaf surface and relative stabilisation in the soil. The efficiency of the nutrient depends on the lesser the particle size the greater the level of nutrient uptake through the plant root system. In addition to this, the nano-particles create a continuous motion state known as 'Brownian Motion' (Jiang, Zhang, Liu, & Sun, 2018). Engineered nanomaterial have been used for seed biofortification for some time and are gaining popularity due to their significant properties and unique functioning style. These particles are small-sized with easy-penetration ability and diffusion in vascular tissues when applied as either foliar or root application to the plants (Torre-Roche et al., 2020). Amongst various methods, seed priming is the most common for fortification of nutritional quality of crops

Table 3. Biofortification through nanotechnology in vegetables.

Crop	Targeted micro-nutrient	Treatment	References
Spinach	Se	SeNPs	Golubkina et al., 2017
Tomato	Se	SeNPs at 10 mg L^{-1} + CuNPs at 50 mg L^{-1}	Hernández-Hernández et al., 2019
	Se	SeNPs at 10–20 mg L^{-1} + CuNPs at 10–50 mg L^{-1}	Quiterio-Gutiérrez et al., 2019
	Se	SeNPs at different conc. (1,5,10 & 20 mg L^{-1})	Morales-Espinoza et al., 2019
Broccoli	Se	Nano-selenium particles (100ppm)	Vicas et al., 2019
Coriander	Ti (B, Ca, Fe, K, Mg, Mn & Zn)	Titanium dioxide NPs (hydroponics)	Hu et al., 2019
Watermelon	Ag	AgNPs seed priming at 96 hours	Acharya, Jayaprakasha, Crosby, Jifon, & Patil, 2020
Lettuce	Cu	CuNPs at 0.2–300 mg L^{-1}	Pelegrino et al., 2020
Green onion	Cu	CuNPs at 75–600 mg Kg^{-1}	Wang et al., 2020
Green pea	Zn	ZnNPs at 100 mg L^{-1}	Skiba, Michlewska, Pietrzak, & Wolf, 2020
Cucumber	Se	SeNPs at 25 mg L^{-1}	Shalaby et al., 2021
Chicory	Se	SeNPs at 40 mg L^{-1}	Abedi, Iranbakhsh, Ardebili, & Ebadi, 2021
Eggplant	Zn	ZnNPs at 40 mg kg^{-1}	Semida et al., 2021
Bell pepper	Cu, Se	CuNPs at 100–500 mg L^{-1}, 10–50 mg L^{-1}	Gonzalez-García et al., 2021

(Paparella et al., 2015). Numerous achievements through nano-technology for quality improvement had enlisted in Table 3.

Biofortification through conventional breeding approach

From the previous four decades, conventional breeding methods predominately focused on enhancing the yield traits and developing resistant cultivars. This unidirectional approach ultimately resulted in reduced nutritional value in existing varieties. The mean concentration of minerals in dry matter of various plant species has declined including iron, magnesium, and copper (Roriz et al., 2020). The conventional breeding approach is one of the easiest and convenient strategies to enhance the phyto-nutritional traits of vegetable crops. There is a need to identify the nutrient rich cultivars within the existed germplasm so that targeted nutrients can be incorporated into the crop to enhance the nutritional status (Gomathi et al., 2017). On a worldwide platform, major research programs have been initiated for some time on biofortification of vegetable crops such as BioCassava, HarvestPlus through conventional breeding approach (Garg et al., 2018). A few important biofortified vegetable crop varieties are illustrated in Table 4

From pre-historic era, alteration in domestic crop plants has been performed to make conventional breeding methods a social mean (Meyer, Duval, & Jensen, 2012). The conventionally developed biofortified foods are cost-effective and have wider acceptance among consumers (Meyer et al., 2012) than transgenic plants (Bouis & Saltzman, 2017). Bouis and Saltzman (2017) opined that conventional approach is more feasible means of biofortification of crops than the agronomic approach as they are not dependent on supplementation through synthetic fertilisers (Edward Marques, Darby, & Kraft, 2021). However, numerous limitations of conventional breeding can become obstacles in biofortification programs as it depends on the standard diversity in the crop-targeted gene pools. It would be applicable only if the target traits are present among the available gene pool (Jha and Warkentin, 2020). The conventional approaches could not exploit the ancillary gene pool which otherwise could be helpful to recognise the valuable nutritional traits (Coyne et al., 2020). The

Table 4. Biofortified varieties developed through conventional breeding approaches.

Crops	Targeted nutrients	Varieties	References
Cassava	Vit-A	TMS 01/1368-UMUCASS-36, TMS 01/1412-UMUCASS-37, TMS 01/1371-UMUCASS 38, NR 07/0220-UMUCASS 44, TMS 07/0593-UMUCASS 45, TMS 07/539-UMUCASS 46	Maziya-Dixon, Kling, Menkir, & Dixon, 2000; Chavez et al., 2005
Potato	Fe & Zn	INIA-321 Kawsay	Garg et al., 2018
Cow pea	Fe	Pant lobia-1, Pant lobia-2, Pant lobia-3, Pant lobia-4	Garg et al., 2018
Common beans	Fe	CAB-2, CODMLB-001, CODMLB-032, CODMLV-059, HM-21-7, RWV-1129, RWR-2154, RWR-2245, RWR-2254, MAC-42,MAC-44, RWV-2887, RWV-3006, RWV-3316, RWV-3317, PVA-1438, VCB-81013, cuarentino, Nain-de-kyondo, Namulenga	Garg et al., 2018
Cauliflower	Vit-A	Pusa BetaKesari, Orange Cheddar, Purple Graffiti	Garg et al., 2018, Yadava, Choudhary, & Mohpatra, 2020
Greater Yam	Zinc, Crude protein, Iron, Calcium	Sree Neelima, Da-340	Yadava et al., 2020
Pea	Protein content	Kinnauri, GC 195, Laxton	Singh & Singh, 2020
Sweet potato	Vitamin – A (β-carotene)	Bhu sona, Bhu krishna, SreeKanka, Bhu Kanti, Bhu Ja, Gouri, Sree Retina, Kamala Sundari, CO-5, Indira Narangi, Indira Madhur, Ejumula, Vita, Olympia, Kkota, Zambezi, Twatasha, Kakamega, Naspot120, and Naspot 130	Yadava et al., 2020, Tengali, Sivakumar, Paul, & Kumar, 2021

wild gene pool carries many beneficial traits compared to the domesticated one. However, pre- and post-zygotic reproduction could be one of the hurdles that might lead to the transfer of undesirable traits from the wild gene pool to targeted crops (Jewell et al., 2020). In the current scenario, wild relatives within the native range have not been properly utilised, due to a lack of *in situ* conservation of wild accessions (Von Wettberg, Chang, Ba¸sdemir, & Singh, 2018). Another limitation of the conventional breeding approach is long duration of developing cultivars and to some extent cost involved for introgression of target traits into desired cultivar on account of extensive selection procedures (Glenn et al., 2017). Several biotechnological tools can help to overcome the limitations of conventional breeding and hastens the process of developing the improved cultivars.

Marker assisted breeding

Maker assisted selection combined with conventional breeding accelerates the breeding programme by minimising the time required for developing any improved cultivar. Kalia et al. (2018) introgressed 'Or' mutant gene into early and mid-maturing groups of cauliflower through marker assisted breeding and identified few lines carrying β-carotene content about 10–12 ppm. Additionally, attempts were made to introgress 'Pr' gene to level up anthocyanin content in diverse cauliflower cultivars such as Pusa Snowball K-1 and Pusa Snowball K-25 via marker assisted back-cross selection (Kalia & Singh, 2018).

Biofortification through biotechnological tools

Biotechnological techniques are powerful methods for generating biofortified crops globally to combat vitamin and mineral deficiencies (Chaudhary et al., 2019). Genetic engineering emerges as a valid alternative to conventional breeding and other approaches to increase the concentration and bioavailability of the micro-nutrients in the edible portion of the crop. The genetic methods are applicable where sufficient variation is lacking either in the crop itself or among the genotypes for desirable traits within the species (Winkler, 2011). The transgenic approach is an effective and feasible means of genetic fortification by introducing the genetic information directly to the plant genome with a specifically targeted micro-nutrient which is not naturally available in the crop plant (Bouis, Saltzman, Low, Ball, & Covic, 2017). The foremost requirement of genetic engineering is to distinguish and identification of gene function metabolism that help to reduce the availability of anti-nutrient factors and enhance micronutrient content through the promoter substances by ensuring their bio-availability (Athar, Khan, Pandey, Yilmaz, & Gezgin, 2020). In addition, genetic modification can ensure the re-distribution of micronutrients among the plant tissue to increase their concentration and reconstruct the selective pathways to progress the effectiveness of the particular biochemical processes in the edible plant parts of the crop (Hefferon, 2015). CRISPR/Cas9 based genome editing is an advanced technology that enables the loss or gain of the function of a particular gene to produce the plant with desirable traits (Yin, Gao, & Qiu, 2017). In an attempt to enhance the nutritional level of tomato from *Solanum pimpinellifolium*, high-density linkage map has been constructed. A next-level linkage map had used to recognise the QTL responsible for mineral content regulation in the tomato plant (Capel et al., 2017). Zouari et al. (2014) reported 25 quantitative trait loci to explain the involvement of genetic variations of mineral content in tomato fruits. The folic acid content had improved with the over expression of the enzyme 'GTP cyclohydrolase-I' (precursor of folic acid) which is mainly involved in the pteridine synthesis (de la Garza et al., 2004). Ye et al. (2015) identified the participation of the transcription factor through transcriptome profiling that involved in the biosynthesis pathway of ascorbic acid. About 26,397 genes expression pattern has been detected, together with the genes concerned with ascorbic acid. The genes involved in the biosynthesis of ascorbate were identified in tomato as mentioned in the report of Sacco, Raiola, Calafiore, Barone, and Rigano (2019). All these studies led to identify two vital enzymes namely, L- ascorbate oxidase and pectin methylesterase which are involved in the accumulation of ascorbic acid in tomato fruits (Table 5). A genome-wide association study was initiated to identify a quantitative-trait-locus related to the accumulation of ascorbate content denoted as *TAF9*, co-localised with transcription factor "*S1b HLH59* (Ye et al., 2019). Vitamin C content was enhanced in tomato through over-expression of GDP-mannose 3',5'-epimerase, *DHAR* and coexpression of arabinono-1,4-lactone oxidase, myo-inositol oxygenase-2 and GDP-mannose pyrophosphorylase (Cronje et al., 2012; Haroldsen, Chi-Ham, Kulkarni, Lorence, & Bennet, 2011; Zhang et al., 2018).

Metabolic engineering allows the over expression of two folate production genes which have increased these contents in tomato fruits (Hanson & Gregory, 2011). The iron content in lettuce was increased introducing a ferritin gene from soybean (Goto, Yoshihara, & Saiki, 2000). The folate accumulation was increased to the extent of 12 folds in potato by targeting *FPGS, HPPK/DHPS* genes via metabolic engineering (Lepeleire, Strobbe, Verstraete, Blancquaert, & Der Straeten, 2017). Similarly, in sweet potato, iron uptake had improved by using embryogenic suspensions and *A. tumefactions*

Table 5. List of biofortified crops using genetic modification techniques.

Crop	Target gene(s)/tissue(s)	Edit tool	Enhanced quality trait(s)	Reference
Tomato	*SlNCED1*	RNAi (fruit specific)	Reduction in ABA; increased beta-carotene, pectin and lycopene	Sun et al., 2012
	Delila & Rosea1	-do-	*anthocyanin*	Maligeppagol, Chandra, Navale, & Kumar, 2013
	Lyc 7.1 & Lyc 12.1	Linkage mapping	Lycopene	Kinkade & Foolad, 2013
	Beta-carotene hydroxylase & ketolose	-do-	Up-regualtion of inherent cartoenogenic genes	Huang, Zhong, Liu, Sandmann, & Chen, 2013
	SlANT1	*TALEN & CRISPR/Cas9*	Anthocyanin	Cermak, Baltes, Cegan, Zhang, & Voytas, 2015
	GME, GaLDH & GCHA or ADCS	Transgenic	Increased ascorbic acid	Gomathi et al., 2017
	CYC-B	*CRISPR/Cas9*	Lycopene (110–500% increase)	Zsogon et al., 2018
Potato				
	VInv	TALENs	Reduced acrylamide and resistance against CIS	Clasen et al., 2016
	VInv, StAS1 & StAS2	RNAi silencing	Reduction in acrylamide content	Zhu et al., 2016
	GBSS	CRISPR/Cas9	Altered starch quality	Andersson, Saltzman, Virk, & Pfeiffer, 2017
	FPGS, HPPK/DHPS	Metabolic engineering	Folate in tubers (12 time higher)	Lepeleire et al., 2017
	AtCGS, *StMGL*	Transgenesis and endogenous, RNAi-mediated gene silencing, qRT-PCR	About 2-fold increase in tuber AtCGS: methionine biosynthesis; StMGL: prevent methionine degradation	Kumar & Jander, 2017
	AmA1, Boxla, Boxlla & Boxalla-2, & tar-1	Transgenesis	Increased protein content	Gomathi et al., 2017
	WRI1, DGAT1, OLEOSIN	Transgenesis	Around 100-fold increase in tuber TAG	Liu et al., 2017
Sweet Potato				
	IbMYB1, npt II	qRT-PCR	Increased anthocyanin, carotenoids, and antioxidant activity	Park et al., 2015
	IbOr, npt II, Embryogenic Callus, *A. tumefaciens* EHA105	Holdase chaperone activity, BiFC	Carotenoid	Park et al., 2016
	IbVP1, hpt, Embryogenic suspension, *A. tumefactions LBA4404)*	SA, RT-PCR, qRT-PCR	Enhanced Fe uptake, enlarged root system	Fan et al., 2017
	Two starch biosynthetic genes:-*IbGBSSI and IbSBEII*	CRISPR/Cas9	Improved starch quality;*IbGBSSI* reduce amylose;*IbSBEII* increase amylose	Wang, Zhang, & Zhu, 2019
Cauliflower				
	Or gene	AFLP, SSR	Pro-vitamin-A (β- carotene)	Li & Garvin, 2003; Kalia, Soi, & Muthukumar, 2016
Cassava				
	Erwinia crtB phytoene-synthase gene, & Arbidoopsis 1-deoxyxylulose-5-phosphate synthase	Transgenic	Pro-vitamin-A	Sayre et al., 2011
	EFA1 gene	Transgenic	Iron content	Sharma, Aggrwal, & Kaur, 2017

LBA4404 targeting *IbVP1, hpt* genes (Fan et al., 2017). The expression of genes like *HMT, SAMT* and *S3H* could enhance the iodine concentration in tomato fruits (de Oliveria et al., 2019). Apart from this, Cathie Martin a British researcher with his co-worker Jonathan Jones has developed a purple-colour high anthocyanin-rich tomato through genetic engineering. Where two transcription factor encoding genes i.e. *Delila* (*Del*) and *Rosea-1* (*Ros1*) of snapdragon flower, were expressed in a tomato cultivar named MicroTom by using RNAi technology (Butelli et al., 2008).

Many health and political issues restrict the commercialisation of transgenic crops, particularly in developing countries (Ansari et al., 2020). Though, in Philippines, golden rice was commercialised in 2019 (Malik & Maqbool, 2020).

SWOT for biofortification in vegetable crops

The traditional crop cultivars containing good nutritional traits and rich in chemical compounds can be used further in nutrient enhancement programs (Renna, 2018). The limited knowledge, cultivation, marketing, and inadequate regulation of biofortification initiatives and their implementation are the vital aspects that need to be solved (Matsungo, Musamadya, Tagwireyi, Takawira, & Chapoto, 2018). They should require specific activities to make aware consumers and growers about the benefits of biofortified crops (Renna, 2018). To boost up the biofortification programs, the policy system can open up the ways for getting more opportunities. Biofortified crops can be utilised by the vibrant food industry to produce nutritious products to make benefit from the potential market. The lack of local and international standards is a major threat influencing biofortification programs. There is a need to resolve consumers fear about GMO foods and also educating them to understand the basic difference between biofortified and genetically modified crops along with their perception of poor organoleptic properties of biofortified products as compared to traditional cultivars (Matsungo et al., 2018).

Organisation involved in bio-fortification to improve nutritional status in agricultural and vegetable crops

Several national and international agencies are involved in enhancing nutritional quality of different crops and to unravel the malnutrition status in several countries. Among these, the prominent ones are CGIAR (Consultative Group on International Agricultural Research), CIAT (Centro Internacional de Agricultura Tropical), CIMMYT (Centro Internacional de Mejoramiento de Maızy Trigo), FAO (The Food and Agriculture Organization), FFI (The Flour Fortification Initiative), GAIN (Global Alliance for Improved Nutrition HarvestPlus initiative), ICCIDD (The International Council for the Control of Iodine Deficiency Disorders), IFPRI (The International Food Policy Research Institute), IRRI (International Rice Research Institute), IZINCG (The International Zinc Nutrition Consultative Group), MI (The Micronutrient Initiative), UNICEF (Unite for Children), WFP (United Nations World Food Programme), WHO (The World Health Organization) & ZTF (The Zinc Task Force) (Plus, 2012 & Andersson et al., 2017).

Conclusions

In the current situation, hunger and malnutrition is 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 approach because the improved cultivars could be utilised in the long term, and do not have any health issues than of agronomic and transgenic approaches. Enhancing the biofortification of nutrients and other quality traits in vegetable crops require strengthening of germplasm pool. A close collaboration between nutritionists and breeders is required to initiate crop specific breeding program including indigenous vegetables.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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