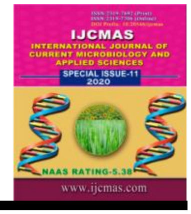
Int.J.Curr.Microbiol.App.Sci (2020) Special Issue-11: 2499-2511

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Special Issue-11 pp. 2499-2511**
Journal homepage: http://www.ijcmas.com



Original Research Article

Biofortification: Enriching Vegetable Crops with Nutrients to reduce Global Hunger

Rehan[1*], Ankita Sharma[1] and Pragya Singh[2]

[1]Department of Horticulture, Jawaharlal Nehru Krishi Vishwa Vidhyalaya, Jabalpur, Madhya Pradesh, India
[2]Department of Horticulture, R.V.S.K.V.V, Gwalior, M.P., India
**Corresponding author*

Keywords

Biofortication, Agricultural practices, Crops and nutrients

ABSTRACT

Traditional agricultural practices enhanced nutritional content in plant food measurably but biofortification augments bio-availability of food with beneficial components using different techniques to provide a continuing and sustainable strategy. Fortification is the practice of deliberately increasing the content of an essential micronutrient, i.e. vitamins and minerals (including trace elements) in a food, so as to improve the nutritional quality of the food supply and provide a public health benefit with minimal risk to health. Biofortification differs from conventional fortification in that biofortification aims to increase nutrient levels in crops during plant growth rather than through manual means during processing of the crops.

Introduction

Indian agriculture is bestowed upon with various cropping seasons, agro-climatic zones and a mega-biodiversity where all crops can survive. The biggest challenges are inadequate food, hunger and malnourishment due to the ever-increasing World population. Vegetables are a rich reservoir of nutrients and as per the recommendation made by ICMR, an average man with vegetarian or non-vegetarian food habits should consume 300 g day^{-1} capita^{-1} comprising of 125 g leafy vegetables, 100 g tubers and 75 g of other vegetables. Malnutrition can be overcome by using nutraceauticals which assists in achieving nutritional security by using micronutrient interventions. Biofortification is an economical approach of nutrient fortification into food crops providing more nutritious food to a large population by availing a few land resources. 'Designer food' can be produced from biofortified crops having a higher content of nutrients subsequently by genetic modification and classical breeding.

Micronutrient deficiencies lead to a global problem of malnourishment of the population due to inadequate availability of vitamins and other micronutrients such as iron, zinc, calcium, iodine and selenium which affects human health. Biofortified varieties enhance the yield potential and nutritional security due to the enrichment of nutritional components in the crops. Traditional agricultural practices enhanced nutritional content in plant food measurably but biofortification augments bio-availability of food with beneficial components using different techniques to provide a continuing and sustainable strategy.

Fortification is the practice of deliberately increasing the content of an essential micronutrient, i.e. vitamins and minerals (including trace elements) in a food, so as to improve the nutritional quality of the food supply and provide a public health benefit with minimal risk to health. Biofortification differs from conventional fortification in that biofortification aims to increase nutrient levels in crops during plant growth rather than through manual means during processing of the crops.

According to WHO, Malnutrition includes both under nutrition as well as over-nutrition and refers to deficiencies, excesses or imbalances in the intake of energy, protein and/or other nutrients. Under nutrition manifests in four broad forms: wasting, stunting, under weight, and micronutrient deficiencies. India loss in GDP due to malnutrition is US$ 12b (IFPRI 2016) Despite a fast-growing economy and the largest anti-malnutrition programme, India has the world's worst level of child malnutrition. As per the Global Nutrition Report 2018, with 46.6 million stunted (low height for age) children, India tops the list of countries followed by Nigeria (13.9 million) and Pakistan (10.7 million). India also accounts for 25.5 million children having low weight for height, followed by Nigeria (3.4 million) and Indonesia (3.3 million). Nearly half of all under-5 child mortality in India is attributable to under nutrition.

According to UNICEF, one in three malnourished children in the world is Indian. It is estimated that reducing malnutrition could add some 3% to India's GDP. (Singh, 2019)

Hidden hunger is a lack of vitamins and minerals. Hidden hunger occurs when the quality of food people eat does not meet their nutrient requirements, so the food is deficient in micronutrients such as the vitamins and minerals that they need for their growth and development. Major micronutrient deficiencies include zinc, iron, iodine, folic acid, vitamin D, and Vitamin E (Biesalski HK and Birner, 2018). Nutritional supplements are one solution, but these are expensive.

By enrichment of staple food with required micronutrients is one way to fight this hidden hunger. Several agricultural strategies like fertilization with micronutrients, breeding for higher micronutrient status of crop variety, making transgenic and biofortification can noticeably contribute in elevation of hidden hunger. According to estimates, around 30-40% of preschool children and pregnant women in developed countries suffer from iron deficiencies (Lucca *et al.,* 2006).

The most prevalent condition caused by iron deficiency is Anemia which results in complications such as fatigue, hair loss and restless leg syndrome. Several untreated deficiencies due to iron leads to morbidity and death (Dosman *et al.,* 2012; Miller, 2013). Zinc is also an essential nutrient for the body as it improves the immune system (Maret and Sandstead, 2006). Deficiency of zinc leads to night blindness, weight loss, poor appetite, dermatitis and poor immunity. Since zinc cannot be stored in the body like fat soluble vitamins, therefore, there is a constant requirement of zinc enriched diet (Frassinetti *et al.,* 2006).

Biofortication

Biofortification is derived from the Greek words where *'bios'* means 'life' and *'fortificare'* means 'to make strong'. Biological Fortification is an imminent and a promising approach of nutritionally elevating food crops with increased bio-available mineral content to address the negative impact on health and economy due to vitamin and mineral deficiencies in humans. It consists of breeding new varieties of staple food such as cassava, sweet potato, potato and beans which are often deficient in minerals using modern biotechnological techniques, conventional plant breeding and agronomic approaches. It is a complementary and a feasible means of reaching remote regions, which girdles around a majority of the malnourished vulnerable population having limited access to a diverse diet, by combining micronutrient trait with other agronomic and consumption traits.

Borg *et al.,* 2009 reported that biofortified crops also improved the efficiency of growth in soils depleted with mineral composition. Some unconventional strategies comprise of identification of plant varieties with enhanced phytonutrient levels within germplasm collections or existing cultivars. This is advantageous in identifying lines which are acceptable by consumers or are a potential donar (Hare, 2005) (Fig. 1).

Necessity and socio-economical aspect

Human beings require an adequate amount of nutrients to live a productive and healthy life. These nutrients play a critical role in the development of physical and mental health. Agricultural and horticultural produce are a primary source of micronutrients but are mostly deficient of essential elements, leading to disabilities, sickness, increased morbidity, impaired development, diminished livelihood, and reduced national and socio-economic development. A large number of people around the World are chronically undernourished, and deprived of commercially marketed fortified foods, mainly women and children, inspite of the advancements. This is an elite technique to overcome hidden hunger by the addition of desired minerals to improve the quality of crops. Nutritional targets for biofortification includes elevated mineral content, improved vitamin content, increased essential amino acid levels, better fatty acid compositions, and heightened antioxidant level in crops. Biofortified crops provide a low-cost way of reaching people having poor access to formal and health-care systems. Bouis *et al.,* (2011) summarised that the biofortified staple food might be unable to furnish as high level of minerals and vitamins per day as the supplemental or industrially fortified food products, but assist in enhancing the daily adequacy of micronutrients intake among the individuals throughout their life cycles.

Objectives of biofortification

To develop vegetable crops containing highly available micronutrients such as iron, zinc and vitamin A for preventing global deficiency of these nutrients.

To screen for biofortification of vegetable crops from existing germplasm.

To study the efficacy of mineral nutrients.

Mechanism and methods of biofortification

Biofortification can be successfully implemented by adopting these strategies suggested by Jena *et al.,* 2018 are:

First, Successful Breeding methods- High nutrient density combined with high yield and profitability.

Second, Efficacy must be demonstrated- the micronutrient status of humans should improve after consumption of biofortified varieties. Sufficient nutrients are retained in cooking and processing.

Third, Biofortified crops must be adopted by the farmers and consumed by the malnourished population.

Biofortification through agronomical methods

Agronomical biofortification employs techniques of fertilizer application or fertifortification through seed treatment, foliar application and use of organic manure to increase nutraceautical values as these are less expensive and quick methods for elevating mineral contents in various vegetables. White and Broadley (2003) depicted that the degree of success in agronomic biofortification is proportional to the mobility of mineral element in the soil as well as in the plant. A major constraint for agronomical biofortification is lowered phytoavailability of nutrients in the soil. Therefore, application of mineral fertilizers to enhance the mobilization and solubalisation of macro and micro nutrients as organic amendments, inorganic fertilizers or through foliar application (Jena *et al.,* 2018). The uptake of acidifying fertilizers such as ammonium nitrate, urea etc is inhibited due to high pH of the soil. Several methods such as crop rotation, intercropping, introducing beneficial soil organisms along with improving the soil infrastructure helps in enhancing food production, sustainability and nutritional security of India's vegetarian population (Mallik and Maqbool, 2020).

Most convenient micronutrient for agronomic fortification are Selenium, Zinc, Iodine, Copper etc. Application of nutrients to fortify plants is done through foliar spray of $ZnSO_4$ or through soil application of iodide or selenate. Mycorrhizal association increases the uptake and regulation of micronutrients like Se, Fe, Cu and Zn in crops. Studies have shown that sulphur content in onion can be increased by sulphur oxidizing bacteria. To degrade the organic matter in the soil, the plant releases some enzymes and organic acid along with some signalling compounds such as flavonoids so as to attract certain microbes to provide protecton against biotic and environmental stresses and increase the nutrient acquisition (Wood and Baudron, 2018: Lye *et al.,* 2020). The plant growth promoting bacteria releases hormones, secondary metabolites and antibiotics in the soil aids in restoring soil quality, nutrient assimilation and improve the uptake of water by the plants for proper growth and development (Goicoechea and Antolin, 2017; Backer *et al.*, 2018). Another practice for soil amendment is tillage which promotes better growth of the plant through proper mixing of the soil with organic matter and inorganic fertilizers in the soil. This helps to meet the desired nutritional value of the crop and also helps in maintaining the fertility and productivity of the soil even under adverse conditions (Patil *et al.,* 2015).

Tomato

Plants can tolerate high level of iodine, stored in vegetative tissues and fruits in ample amount for human diet thus are an excellent crop for iodine-biofortification program. Use of an environmentally safe new generation organic fertilizer "Riverm" to enrich Solanaceous crops tomato, chilli, brinjal with zinc (Bouis *et al.,* 2011).

Potato

Foliar application of zinc improves Zn concentration in potato was reported by White *et al.*, (2012).

Amaranthus

Amaranthus gangeticus leaves are a rich source of Zn, Fe, Ca, Mg and Cu. *Spirulina platensis*, a microbial inoculant was used as a biofortifying agent to enhance the iron level of crop (Kalpana *et al.*, 2014).

Se-enriched *S. pinnata* used for enriching broccoli, carrots and onions with healthful forms of organic-Se by foliar application of a solution of 77Se(IV).

Using conventional breeding methods

Biofortification is a practice which has the potential to augment the density of micronutrients in staple food through selective breeding approaches or through biotechnological approaches. (Garg *et al.*, 2018) Conventional breeding practices help in improving the concentration of β-carotene, caroteniods, amino acids, amylase, carbohydrates and other minerals through making proper selection of breeding material to increase nutritional efficiency (Gregorio *et al.*, 2000).

This is an ecologically and economically stable process of crop development for nutraceautical values of the food. Collard and Mackill, 2008 have concluded that biotechnological approaches such as marker assisted selection, employed along with breeding programs remarkably increases the success breeding approaches in strengthening the nutritional quotient of the crop.

Steps in biofortification through breeding are as follows (Fig. 2):

Identification of target population.

Setting of the nutrient target levels.

Screening of germplasm.

Developing biofortified varieties.

Testing the performance of new crop varieties

Measuring nutrient retention in crop

Evaluating the absorption of nutrient.

Developing strategies for disseminating seeds

Promoting marketing and consumption of biofortified food

Improving nutritional status of targeted population.

Potato: Kufri Neelkanth

Antioxidant rich table purpose variety. Tubers of the hybrid are dark purple black, ovoid in shape with medium deep eyes, cream flesh, good storability, medium dry matter (18%) and medium dormancy with excellent flavour. Suitable for growing in North Indian plains developed from CPRI, Shimla. (Source: icar.org.in). A Disease resistant triploid clone was developed by International Potato Center (CIP) and Harvest Plus by crossing the diploid Andean landrace Potato with high zinc and iron content through three cycles of recurrent selection.

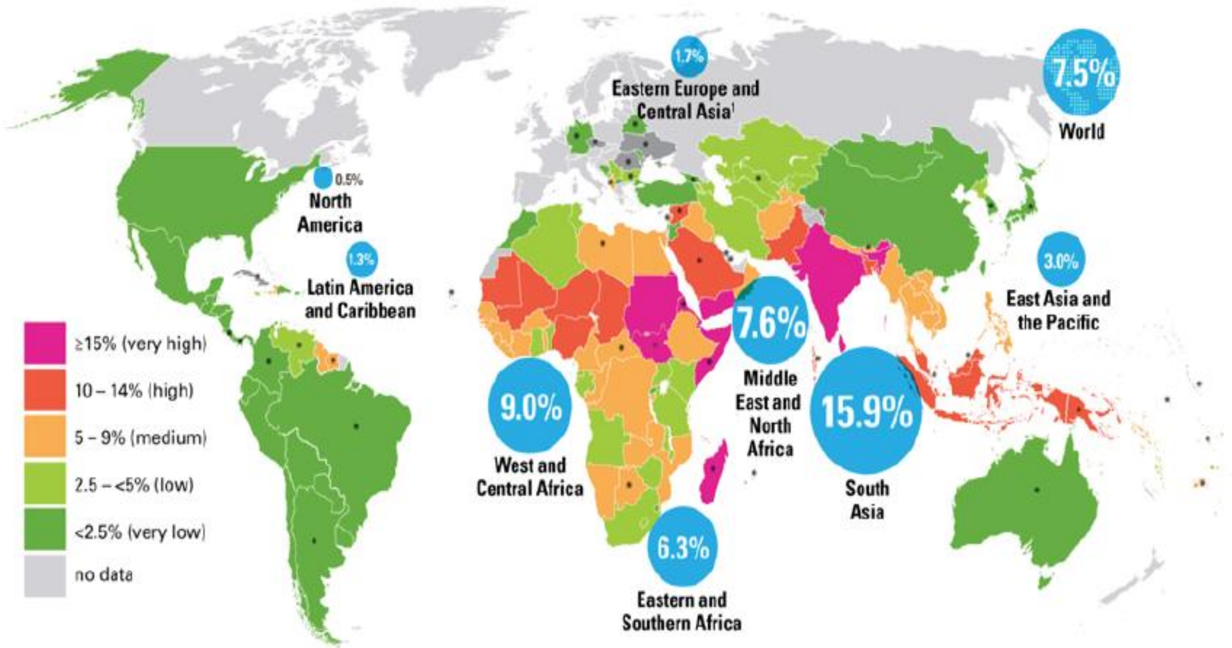
Brinjal variety 'Pusa Safed Baigan 1'

Released by IARI in 2018. White coloured oval fruit rich in total phenol content and high antioxidant property.

Cauliflower: Pusa Beta Kesari 1 (Pure line variety)

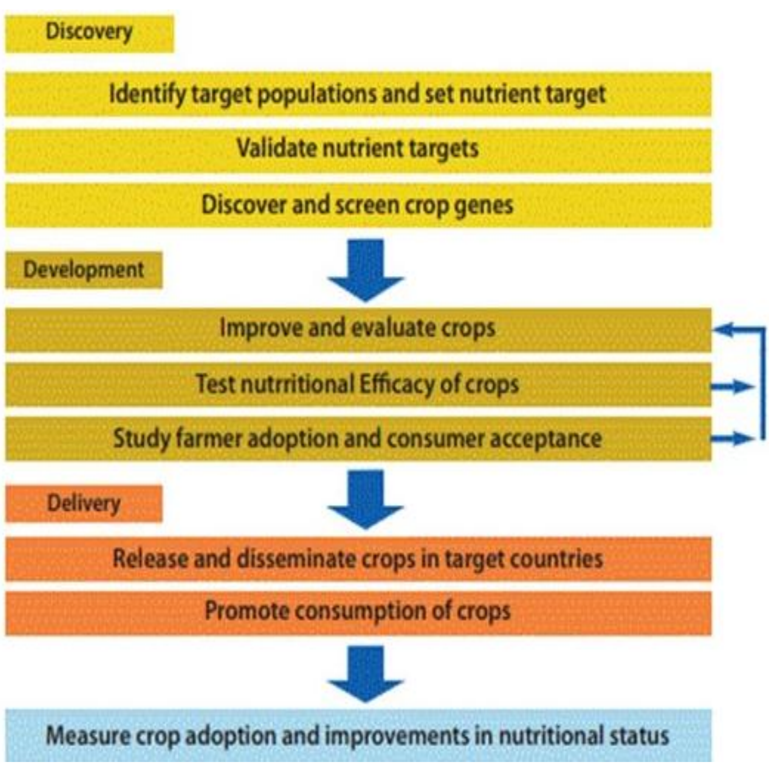
Country's first biofortified cauliflower. Contains high β-carotene (8.0-10.0 ppm) in comparison to negligible β-carotene content in popular varieties. Developed by ICAR, New Delhi in 2015.

Fig.1



Source: UNICEF DATA (2018)

Fig.2 Pathway suggested by harvest plus



Source: Bouis *et al.*, (2017)

Table.1 Source of Nutrients from vegetables

Nutrient	Source
Carbohydrate	Sweet potato, Potato, Cassava
Protein	Pea, lima bean, French bean, cowpea
Vitamin A	Carrot, spinach, pumpkin
Vitamin B_1	Tomato, chilli, garlic, leek, pea
Vitamin C	Chilli, sweet pepper, cabbage, drumstick
Calcium	Hyacinth bean, amaranthus, palak
Iron	Amaranthus, palak, spinach, lettuce, bitter gourd
Phosphorous	Pea, lima bean, taro, drumstick leaves
Vitamin B_5	Palak, amaranthus, bitter gourd, pointed gourd
Iodine	Tomato, sweet pepper, carrot, garlic, okra
Sodium	Celery, green onion, Chinese cabbage, radish

Source: Food and Nutrition Board, 2013

Table.2 Sweet potato

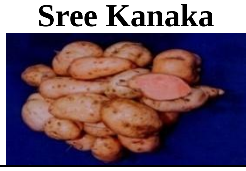
Variety	Character	Institute	Year	source
Bhu Sona 	High β-carotene (14.0 mg/100g) • Tuber yield: 19.8 t/ha • Dry matter: 27.0-29.0% • Starch: 20.0% • Total sugar: 2.0-2.4% • Adaptation: Odisha	ICAR-Central Tuber Crops Research Institute, Thiruvananthapuram, Kerala	2017	icar.org.in
Bhu Krishna 	High anthocyanin(90mg/100g) • Tuber yield: 18.0 t/ha • Dry matter: 24.0-25.5% • Starch: 19.5% • Total sugar: 1.9-2.2% • Salinity stress tolerant • Adaptation: Odisha	ICAR-Central Tuber Crops Research Institute, Thiruvananthapuram, Kerala	2017	icar.org.in
Sree Kanaka 	Early maturing (75-85 days) Cylindrical tubers with dark orange flesh colour High beta carotene.	CTCRI, Thiruvanantpuram		www.ctcri.org

Table.3 Carrot

Variety	Character	Institute	Year	source
Pusa Rudhira 	Has higher level of Carotenoid (7.14mg) & Phenol (45.15mg)/ 100g Possess antioxidant property self core red coloured with delayed bolting.	IARI, New Delhi	2008	www.iari.res.in
Pusa Asita 	self black coloured roots Late bolter. Rich source of anthocyanin.	IARI, New Delhi	2008	www.iari.res.in

Table.4 Raddish**:** Pink and purple fleshed radish varieties were released by IARI

Variety	Character	Institute	Year	source
Pusa Gulabi 	First Pink fleshed variety High in total caroteniods, anthocyanin and ascorbic acid content. Grows exceptionally well in heat of summer. Medium root size, cylindrical shape	IARI, New Delhi	2013	www.iari.res.in
Pusa Jamuni 	first purple fleshed nutritionally rich variety high in anthocyanin & ascorbic acid content.	IARI, New Delhi	2012	www.iari.res.in

Table.5 Donar parents having nutraceautical values in different vegetable crops

Crop	Trait	Donor(s)
Tomato	High ascorbic acid	*S. pimpinellifolium*, Double Rich
	Pro-vitamin A (beta carotene)	Crimson and Caro Red
Potato	High protein content	*S. phureja*, *S. vernei*
Pea	Protein	GC 195, Kinnauri, Laxton
Pumpkin	Carotene	Golden Delicious
Carrot	Vitamin- A	Pusa Meghali
Pepper	Carotene	Douxed Alger

Source: Yadav *et al*. (2017)

Table.6 Transgenic research in potato for quality improvement

S.No.	Quality trait	Gene	source
1.	Amino acid-rich storage protein	*AmA1* *tar1* (tarin) *Boxla, BoxIIa, BoxlaIIa 2*	*Amaranthus hypochondriacus* *Colocasia esculenta* *Bertholletia excels*
2.	High amylose starch	*SBE I* antisense	Potato
3.	Carbohydrate engineering	*SUSI* (sucrose synthase)	Potato
4.	High tuber galactose	*stUGE451 stUGE5I*	Potato
5.	High tuber fructose	*xylA* (glucose isomerase)	*Thermus thermophilus*

(Source: Pandey *et al*. 2005)

Table.7 Engineering for quality improvement in tomato

Fruit Trait	Targeted Gene
Carotenoid content	Dxs, CrtB, CrtI, CrtY, PSY-1, CRY-2, CYC-B, LCY-B, CHY-B, DET1, COP1LIKE, CUL4, FIBRILLIN, Spermidine synthase, PG
Flavonoid content	CHI, CHS, CHI, F3H, FLS, MYB12, STS, CHR, FNS-II, Del, Ros1
Ascorbic acid content	GaLDH, GME, GCHA and/or ADCS
Nutritional value	Crt1, Samc

(Source: Matteo *et al.* 2011)

Cowpea

Two early maturing, high iron and zinc fortified varieties Pant Lobia-1 and Pant Lobia-2 have been developed by GBPUAT, Pantnagar.

Limitations in selective breeding approaches

Low heritability.

Lack of genetic diversity for micronutrients

Lack of sufficient variation among genotypes for desired trait.

Linkage drag

Use of transgenic techniques

Genetic engineering techniques utilize an unlimited pool of genes to produce new cultivars through transfer of desirable characters from one organism to another to develop elite cultivars, thereby improving its value.

GE techniques have conquered new phases of multiple gene transfer which helps in enhancing the level of provitamin A, vitamin B9, folate, ascorbate to identify promising lines. Some distinct micronutrients can be naturally produced in crops (Perez-Massot *et al.,* 2013). Now-a-days, micronutrient powders are also available in sachets which can be sprinkled on top of normal food to provide several nutrients. (Jena *et al.,* 2018) Transgenic crops are genetically modified crops in which not only the nutritional quality is enhanced but also provides protection against insect, viruses and pathogens. GE offers valid alternative to increase the concentration and bioavailability of micronutrients in edible crop tissues (Prasad *et al.,* 2015).

Many vegetable crops have been produced through genetic modification by improving traits such as better flavour, reduced bitterness, slow ripening, high nutritional status, seedless fruits, increased sweetness and reduced anti-nutritional factors. (Mallik and Maqbool, 2020)

Carrot

Bio-available calcium content in transgenically modified carrots was found to increase by the Arabidopsis H+/Ca2+ transporter CAX1 thus Ca content reduce the incidence of osteoporosis.

Lettuce

Salad crop rich in Vit A, C, Ca but has low iron content. A soybean ferritin gene has been used to improve Fe content and yield.

Cauliflower

Map based cloning done by isolating copia-like LTR retrotransponson Or gene to regulate caroteniods accumulation and increased the level of β-carotene in mutant orange cauliflower.

Sweet Potato

It is an alternate source of energy, rich in antioxidants, anthocyanin and dietary fibres. The content of lutein and caroteniods was increased by expressing orange IbOr-Ins gene in white fleshed sweet potato.

Cassava

Staple food deficient in Pro-vit A, Iron and zinc. Plants over-expressing a PSY transgene produced yellow-fleshed and high-carotenoid roots. Cyanogen-free cassava was produced by encoding the genes (CYP79D1 and CYP79D2).

Potato

β-carotene rich potato produced by expressing *Erwinia uredovora crtB* gene. Starch, protein and amino acid rich potatoes developed by expressing the genes as mentioned in the table.

Tomato

To increase the content of Lycopene, anthocyanin, flavonols and antioxidant content in tomato the following genes were expressed to produce transgenic tomato.

Potential risks or concerns from use of GM Foods

Alteration in nutritional quality of foods

Antibiotic resistance

Potential toxicity and allergenicity from GM foods

Unintentional gene transfer to wild plants

Possible creation of new toxins

Limited access to seeds through patenting of GM food plants

Threat to crop genetic diversity

Religious/cultural ethical concerns

Concerns for lack of labelling

Concerns of organic and traditional farmers

Fear of the unknown.

Biosafety concerns in the development and commercialization of transgenics

Biosafety refers to policies and procedures adopted to ensure environmental safety during the course of the development and commercialization of the transgenic organisms.

Escape of engineered gene by the gene flow or gene disposal.

Non-target or ecological effects.

Invasiveness or weediness of transgenics. It means tendency of plant to spread beyond the field where first planted.

Creation of super-weeds and super-viruses.

Toxicity and allerginicity to human beings and animals.

Expression of undesirable phenotypic traits.

Genetic erosion of biological diversity.

Biofortification: a cost effective approach

An overall food system will provide the most sustainable means to incorporate essential vitamins and minerals into the diet. Biofortification is a feasible, efficacious, cost-effective sustainable and scalable agricultural-nutrition intervention to deliver essential micronutrients to malnourished rural and vulnerable populations having limited access to diverse diets, supplements or fortified food (Bouis *et al.,* 2017). According to World bank criteria, biofortification is highly cost effective. Biofortification is a revolutionary innovation which can significantly reduce the number of people suffering from hidden hunger and to ensure a healthy and productive life.

The new biofortified crops require meticulous development and complement the existing interventions but it cannot be expected to eliminate nutritional deficiency completely. It is indeed a challenging endeavour to enhance the micronutrient concentration in a sufficient amount and the acceptance of transgenically fortified crop plants by consumers. Nutraceautical biofortified vegetables have great potential to overcome hidden hunger billions of poor people and will help to achieve the ultimate aim of global nutrition and a diverse diet for the world's population using an eco-friendly technique. Biofortification hold great promises in improving the nutritive value of major crops. Using recombinant DNA techniques, the bioavailability of several micronutrients and vitamins can be increased. External fortification of the nutrients has a limited value such as fortified material which is available for the urban population. In developing countries, a large part of population is rural and fortified food is neither accessible nor is affordable for them. Biofortification is a technology of producing major staple crops through seeds which is advantageous in meeting the nutritive requirements of people at an affordable cost.

References

Biesalski HK and Birner R (eds): Hidden Hunger: Strategies to Improve Nutrition Quality. World Rev Nutr Diet. Basel, Karger, 2018, vol 118, pp 161-166.

Borg S, Brinch-Pedersen H, Tauris B, and

Holm PB. 2009. Iron transport, deposition and bioavailability in the wheat and barley grain. Plant and Soil, 325: 15-24.

Bouis, B. Mc Clafferty, J. V. Meenakshi, and W. H. Pfeiffer.Biofortification:2011. A New Tool to Reduce Micronutrient Malnutrition. Suppl. Food Nutr. Bull, 32 (1): 31-40

Bouis H, Saltzman A, Low J, Ball A and Covic N. 2017. An overview of the landscape and approach for biofortification in Africa. African Journal of Food, Agriculture, Nutrition and Development, Vol. 17, No. 2, April 2017

Backer, R., Rokem, J. S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci, E., 2018. Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. *Front. Plant Sci*. 9:1473.

Cercamondi, C. I., Egli, I. M., Mitchikpe, E., Tossou, F., Zeder, C., Hounhouigan, J. D., 2013. Total iron absorption by young women from iron-biofortified pearl millet composite meals is double that from regular millet meals but less than that from post-harvest iron-fortified millet meals. J. Nutr. 143, 1376–1382.

Collard, B. C., and Mackill, D. J. 2008. Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Philos. Trans. R. Soc. Lond. Biol*. *Sci.* 363, 557–572.

Di Matteo, A., M.M. Rigano, A. Sacco, L. Frusciante and A. Barone. 2011. Genetic transformation in tomato: novel tools to improve fruit quality and pharmaceutical production. In: Avarez, M. Genetic transformation. InTech. Pp. 55-80.

Diretto, G., Welsch, R., Tavazza, R., Mourgues, F., Pizzichini, D., Beyer, P., *et al.*, (2007). Silencing of beta-carotene hydroxylase increases total carotenoid and beta-carotene levels in potato tubers. BMC Plant Biol. 7:11.

Dosman, C., Witmans, M., and Zwaigenbaum, L. 2012. Iron's role in paediatric restless legs syndrome–a review. *Paediatr Child Health.* 17, 193–197.

Food and Nutrition Board, 2013. Dietary Reference Intakes. Tables and Application.

Frassinetti, S., Bronzetti, G. L., Caltavuturo, L., Cini, M., and Della Croce, C. 2006. The role of zinc in life: a review. *J. Environ. Pathol. Toxicol. Oncol*. 25, 597–610.

Garg, M., Sharma, N., Sharma, S., Kapoor, P., Kumar, A., Chunduri, V., 2018. Biofortified crops generated by breeding, agronomy, and transgenic approaches are improving lives of millions of people around the world. *Front. Nutr*. 5:12.

Goicoechea, N., and Antolín, M. C. (2017). Increased nutritional value in food crops. *Microb. Biotechnol*.10, 1004–1007.

Gomathi M, Vethamoni Irene P.,and Gopinath P. Biofortification in Vegetable Crops – A Review. Chem Sci Rev Lett 2017, 6(22), 1227-1237

Gregorio, G. B., Senadhira, D., Htut, H and Graham, R. D. 2000. Breeding for trace mineral density in rice. Food and Nutri Bull. 21: 382–386.

Hare T.J.2015. Biofortification of vegetables for the developed world. Acta Hortic, 1106.

Harvest Plus. 2008. HarvestPlus: Breeding Crops for Better Nutrition.

Jena, A.K., Bora, G., Sharma, P., Deuri, R. and Singh, S.P. 2018. Biofortification of Vegetables, Int. J. Pure App.

Biosci. 6(5): 205-212

Kalpana P, Sai Bramari G and Anitha, L. 2014. Biofortification of *Amaranthus gangeticus* using *Spirulina platensis* as microbial inoculant to enhance iron levels. Internat. J. Res. Appl. Nat. Social Sci., 2(3): 103-110.

Lucca, P., Hurrell, R., and Potrykus, I. 2001. Approaches to improving the bioavailability and level of iron in rice seeds. *J. Sci. Food Agric*. 81, 828–834.

Lyu, D., Backer, R., Subramanian, S., and Smith, D. L. 2020. Phytomicrobiome coordination signals hold potential for climate change-resilient agriculture. *Front. Plant Sci*. 11:634.

Malik KA and Maqbool A. 2020. Transgenic Crops for Biofortification. Front. Sustain. Food Syst. 4:571402

Maret, W., and Sandstead, H. H. 2006. Zinc requirements and the risks and benefits of zinc supplementation. *J. Trace Elem. Med. Biol*. 20, 3–18.

Miller, J. L. 2013. Iron deficiency anemia: a common and curable disease. *Cold Spring Harb. Perspect. Med*. 3:7.

Parulekar Y. R., Haldankar P. M., Dalvi N. V., Salvi B. R. and Bhattacharyya T.2019. Nutraceuticals and their Biofortification in Vegetable Crops: A Review. Advanced Agricultural Research & Technology Journal. Vol. III

Pandey, S.K., S.V. Singh and D. Sarkar. 2005. Potato (*Solanum tuberosum*) for sustaining food and nutrition security in developing world. Indian Journal of Agricultural Sciences, 75(1): 3-18.

Patil, S. B., Chitgupekar, S. C., and Patil, B. B. 2015. Modern tillage and integrated nutrient management practices for improving soil fertility and productivity of groundnut (*Arachis hypogaea L*.) under rainfed farming system. *Int. Lett. Natl. Sci.* 2, 1–12.

Perez-Massot, E., Banakar, R., Gómez-Galera, S., Zorrilla-López, U., Sanahuja, G., Arjó, G., 2013. The contribution of transgenic plants to better health through improved nutrition: opportunities and constraints. *Genes Nutr*. 8, 29–41.

Prasad B.V.G., Smaranika Mohanta, S. Rahaman and Prerna Bareily. 2015. Bio-fortification in horticulture crops Journal of Agricultural Engineering and Food Tech, 2350-0263: 95-99.

Singh A. 2019. Childhood Malnutrition in India

Uzogara, S.G. 2000. The impact of genetic modification of human foods in the 21st century: a review. Biotechnology Advances, 18: 179-206.

White, P. J., and Broadley, M. R. 2003. Calcium in plants. Annals of Botany, 92, 487–511.

White P. J., Broadley M. R., Hammond J. P., Ramsay G., Subramanian N. K., Thompson J. and Wright G. 2012. Bio-fortification of potato tubers using foliar zinc-fertiliser. J. Hort. Sci Biotech. 87: 123-129.

Wood, S. A., and Baudron, F. 2018. Soil organic matter underlies crop nutritional quality and productivity in smallholder agriculture. *Agr. Ecosyst. Environ*. 266, 100–108.

World Bank. World Development Report 1993: Investing in Health. Washington, DC: World Bank. 1993.

Yadav D. K., Choudhury P. R., Hossain F. and Kumar D. 2017. Biofortified varieties: Sustainable way to alleviate malnutrition. New Delhi: Indian Council of Agricultural Research.