BIOFORTIFICATION OF VARIOUS VEGETABLE CROPS – A REVIEW

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ABSTRACT:

Around 800 million people are hungry, but many more are suffering from micronutrient malnutrition, also known as "hidden hunger," especially in developing countries. Malnutrition of iodine, vitamin A, iron, and zinc is a major concern. Mineral (Fe, Zn) and vitamin A malnutrition is a major food-related primary health problem among populations in the developing world, including India, where cereal-based diets are common and access to fruits and vegetables is limited. Biofortification of vegetables with vitamins and micronutrients is the current need of the hour for developing countries to combat various health issues. Three main techniques are used for biofortification of vegetable and other staple crops: traditional breeding, agronomic approach, and genetic engineering. These methods have a lot of promise in terms of addressing vitamin and micronutrient deficiency. Several studies have recently surfaced about the production of transgenic crops to boost provitamin A levels in crops such as tomato, potato, sweet potato, beans, and other vegetable crops.

KEY WORDS: Biofortification, Vegetable, Iron, Zinc, Iodine, Selenium, transgenic approaches, nutrition.

INTRODUCTION

Food protection has been a big problem on this planet for many decades. Micronutrient deficiencies result from a monotonous diet consisting of milled cereals with few micronutrients, which currently affects one out of every eight people. Main deficiencies affect about 30% of the population, including 60% of zinc, 60% of iron, 15% of selenium, and 30% of iodine. Micronutrients play an important role in human nutrition, in the prevention and treatment of various diseases, as well as promoting mental and physical health (Barsha Tripathy, 2020). However, most significantly, by 2050, the world population would have surpassed 9 billion people, putting enormous strain on agriculture to feed this population (Hubert *et al.,* 2010). Cereals, which are a major staple food in developing countries, are subjected to extensive processing, such as milling and dehulling, during which the majority of nutrients are lost and the food becomes nutritionally deficient. Though traditional cultivation practises can improve the nutritional content of plant foods to some extent, biofortification was developed as a result of advanced research to combat nutritional deficiencies. It is the practise of adding nutrients to food crops through conventional, agronomic, and transgenic breeding methods. It provides a long-term and sustainable solution to vitamin and nutrient deficiency.

Most horticultural crops, such as cassava, banana, beans, orange sweet potato (OSP), potato, pumpkin, cowpea, and others, have benefited from biofortification. A number of different varieties have been released. It is a promising strategy to tackle micronutrient deficiencies to increase the availability of essential nutrients by either decreasing the level of absorption inhibitors or increasing the level of absorption enhancers in crops through traditional plant breeding and genetic engineering. Biofortification can improve the nutritional quality of staple foods by plant breeding, providing a relatively inexpensive, cost-effective, long-term means of providing more micronutrients to the poor. This strategy would reduce the number of people who are chronically malnourished while also assisting them in maintaining a balanced nutritional status. Furthermore, biofortified foods can easily enter rural areas where commercially sold fortified foods and supplements are scarce. Micronutrient malnutrition affects more than half of the world's population, with developing countries bearing the brunt of the issue (Ortiz-Monasterio *et al.*, 2007). To maintain a balanced lifestyle, humans need a small number of macro-elements, a trace number of microelements (Fe, Cu, Zn, I, and Se), and vitamins, as well as a significant amount of starch, protein, and lipids (Welch, 2002). Provitamin A, Fe, I, Zn, and Se deficiency are all stated to have a high percentage of disease burden and a negative effect on the population (Black *et al.*, 2008; Stein, 2010). It is particularly crucial for poor rural communities who have limited access to a diverse diet, fortified foods, or supplements. It can benefit people by increasing their daily micronutrient intake over the course of their lives. It is especially important for women and children, as they are more susceptible to micronutrient malnutrition. For example, the World Health Organization (WHO) estimates that two billion people suffering from iron deficiency-induced anaemia could be cured by eating biofortified foods, thus promoting food security and alleviating poverty.

The main problem with biofortification is that it should be widely adopted by farmers after variety production. The crop must hit the poor people who are in desperate need of it. Vitamins and micronutrients abound in vegetables, fruits, dairy, and meat products, but they're out of reach for the poor. They depend on a small number of starchy staples (rice, wheat, corn, and potato), making dietary diversity a privilege that poor people cannot afford (Gómez *et al.*, 2013). The magnitude of diseases caused by malnutrition and mineral deficiencies is so great that the World Bank estimated that the combined economic cost of mineral deficiency in developing countries could waste up to 5% of their GDP (GDP). Micronutrient and vitamin deficiency have a major effect and burden on society, leading to an increase in infectious disease susceptibility, physical disability, cognitive losses, blindness, and premature mortality.

What Is Biofortification, and How Does It Work?

The growth of micronutrient-dense staple crops (cereals and vegetables) using conventional plant breeding methods, modern biotechnology, and agronomical approaches is known as biofortification. During the growth and development of the plant, the concentration of plant-derived nutrition and vitamins is increased in the edible organ (O'Hare, 2015). This is a method of breeding nutrients into food crops that results in a low-cost, long-term supply of adequate micronutrients. It is a method of enriching edible parts such as grain, straw, roots, fruits, and tubers with micronutrients and vitamins using the right breeding method and biotechnological methods (Bouis, 2000; Saltzman *et al.*, 2013). While biofortified staple foods do not contain as many essential vitamins and micronutrients as industrially fortified foods, they may help to alleviate "hidden hunger" by increasing the regular adequacy of micronutrients absorption by individuals across their lives (Bouis *et al.*, 2011). Biofortification methods include agronomic approaches, conventional breeding, and genetic engineering approaches.

Biofortification Techniques:

Three techniques can be used to achieve biofortification.

1. Agronomic Biofortification

2. Conventional plant breeding

3. Genetic engineering

1. Agronomic Biofortification

Fertilizer is used in this method either as a spray on the leaves or as a soil application (Weng et al. 2008b). The use of foliar application to enhance these nutrients in plant tissue and edible parts was stated to be effective in biofortification of Fe and Zn (Saltzman *et al.*, 2013). Selenium (as selenate), iodine (soil application of iodide or iodate), and zinc are the most important micronutrients for agronomic biofortification (foliar applications of ZnSO4). The foliar application of micronutrients is a simple and fast way to provide micronutrients to plants (Fe, Zn, Cu etc.). AM-fungi improves production and absorption of micronutrients such as copper, iron, and zinc. Sulphur oxidising bacteria increase the sulphur content of onions. Biofortification refers to the various forms of fortification that can substantially increase the number of vitamins and nutrients in a living commodity (edible part), with the vitamins and nutrients being accumulated through the plant's natural physiological processes. However, for tree fruits and nuts, where the juvenile period is even longer (O'Hare, 2015), this would be difficult.

Zinc biofortification of crops

While biofortified staple foods do not contain as many essential vitamins and micronutrients as industrially fortified foods, they may help to alleviate "hidden hunger" by increasing the regular adequacy of micronutrients absorption by individuals across their lives (Bouis *et al.*, 2011). Over 60% of the world's population is deficient in Fe, 30% is deficient in Zn as well as iodine, and 15% is deficient in Se, according to estimates (White and Broadley, 2009). The relationship between tuber Zn concentration and foliar Zn application followed a saturation curve, with a maximum at around the time of the study. 30 mg Zn kg–1 DM at 1.08 g plant–1 foliar Zn application rate Despite a 40-fold increase in shoot Zn concentration following foliar Zn fertilisation with 2.16 g Zn plant–1 relative to unfertilized controls. Fertilizer made from organic materials Reverm can help sweet pepper, eggplant, and tomato plants bio-enrich by Zn, not just because it contains a lot of it, but also because it helps the plant assimilate and absorb the Zn that is already in the soil. Biofortified vegetables have 6.60-8.59 percent more Zn than unfortified vegetables (Yudicheva, 2014).

Selenium biofortification of crops

Se may be taken up by plants in the form of selenite, selenite, or organoselenium compounds. Selenocysteine and selenomethionine are the most common of these compounds, but they cannot absorb colloidal elemental Se from metal selenides (White *et al.*, 2004). Se-enriched S. pinnata is useful as a soil modification for providing healthy types of organic-Se to broccoli and carrots. Biofortification refers to the various forms of fortification that can substantially increase the number of vitamins and nutrients in a living commodity (edible part), with the vitamins and nutrients being accumulated through the plant's natural physiological processes (O'Hare, 2015). Onions and carrots were bio-fortified with 77Se (IV), which was then foliar enriched to 99.7%. The application of selenium had no impact on the yield or oil content of Brassica plants. Se accumulation was found to be higher in the seeds and meal (1.92–1.96 g Se g-1). Biofortification with various micronutrients (Fe, Zn, I, and Se) and vitamins (vitamin A, B, C, E, and K) using breeding methods and genetic engineering is a relatively cost-effective and productive approach for counteracting deficiency in humans and farm animals (Lyons *et al.*, 2004).

Iron-based biofortification

The addition of micronutrients and vitamins to staple foods and vegetables would lead to increased consumption of micronutrients, particularly among the poor, resulting in a reduction in malnutrition (Das *et al.*, 2017) Both the fruits and the vegetative tissues of the tomato store enough iodine for normal human consumption. It is an excellent crop for iodine biofortification because of its resistance to higher levels of iodine. The amount of iodine contained in the fruit of plants treated with 5 mM iodide was enough to cover a daily human intake of 150 g. Spirulina platensis was used as a biofortifying agent to improve the iron status in Amaranthus gangeticus plants when compared to a control.

2. Conventional plant breeding

Conventional breeding is based on natural selection and may be a viable alternative to genetic engineering. It was discovered that newly evolved breeding lines increased the folate content of vegetables such as tomato and potato by twofold (Hanson and Gregory, 2011). It may be possible to biofortify vast quantities of crops and disseminate them around the world using traditional or modern breeding techniques, as well as genetic engineering (Welch and Graham, 2004). More emphasis on yield attributes and resistance breeding in conventional breeding over the last four decades has resulted in poor nutrition in existing varieties. Fe, Zn, Cu, and Mg are examples of minerals whose mean concentration in dry matter has decreased in a variety of plant-based foods. In recent years, traditional breeding has centred on fortification of essential vitamins, antioxidants, and micronutrients. The green movement, which began in the early 1960s, made the world capable of combating food insecurity (Pinstrup Andersen and Hazell, 1985). However, this resulted in a decrease in local production of fruits, vegetables, and legumes, which are the people's primary source of micronutrients (Welch and Graham, 2004).

Plant Breeding for Biofortification of Specific Vegetable Crops:

Sweet Potato: The biofortification program's main goal is to replace low pro-vitamin A white fleshed sweet potato varieties with high pro-vitamin A orange fleshed sweet potato varieties. It is estimated that approximately 250 million pre-school children are at risk of provitamin A deficiency, with a large proportion of pregnant women also at risk. The children are at greater risk as a result of their provitamin deficiency. It causes vision loss, blindness, and an increase in the incidence of diseases such as diarrhoea and measles. In 2016, World Health Organization Diabetes, obesity, some forms of cancer, stroke, inflammation, and other cardiovascular disorders are all linked to poor nutrition (Cömert *et al.*, 2019). Harvest Plus aimed for sweet potato varieties with a 32 g/ g concentration, but varieties with concentrations as high as 100 g/ g are already available. Over the course of 11 weeks, children are given white fleshed potato with no beta-carotene and orange fleshed potato with a beta-carotene concentration of about 100 g/ g in the cooked root. When compared to the control group, the treatment group had more vitamin A in their liver stores. Furthermore, it has been discovered that when boiling orange fleshed sweet potatoes, beta carotene retention is very high, with about 80% of the initial concentration remaining. When dried sweet potatoes were processed, the amount of -carotene or provitamin A changed. Trans-carotene was found to be substantially preserved in hot air cross-flow relative to sun drying, according to Bechoff *et al.* (2009). Drying sweet potato slices in an air oven for 12 hours at 60 degrees Celsius resulted in a 30 percent reduction in total carotenoid (Hagenimana *et al.*, 1999).

Beans: Beans are known as "poor man's meat" because of their low cost as a protein source and high vitamin and mineral content (especially zinc and iron). The need for mineral biofortification in common beans, starting with germplasm screening, inheritance, physiological or bioavailability studies, and ending with the production of new biofortified varieties (Blair, 2013).

3. Genetic Engineering

Biotechnology is an effective biofortification technique that is being used all over the world to tackle the severity of mineral and vitamin deficiency. The recent development in genetic engineering tools and techniques allows for the incorporation of traits that are not possible to achieve by traditional breeding (Chaudhary *et al.*, 2019; Rana *et al.*, 2019). Genetic engineering (GE) is often referred to as a vital technology for potential food, feed, and energy needs. It has been a record since the first large-scale launch of the Flavr-Savr tomato in 1996. Genetically modified (GM) crops (transgenic crops) enable plant breeders to introduce beneficial genes into previously unavailable cultivars, enhancing their value and providing exclusive opportunities to combat viruses, insects, and other pathogens while also improving health benefits and nutritional quality. When there is insufficient variation among genotypes for the desired character/trait within the species, or when the crop is not suitable for traditional plant breeding (due to lack of sexuality), genetic engineering is a viable choice for increasing micronutrient bioavailability and concentration in edible crop tissues. Vegetable breeders are encouraged to use genetic modification to incorporate desired transgenes into novel cultivars, increasing their value. It provides unique opportunities to improve food quality and health benefits. In vegetable crops, genetic engineering is used to enhance characteristics such as flavour, nutritional status, bitterness, slow ripening, seedless fruit, increased sweetness, and anti-nutritional factors.

The purpose of using genetic engineering to biofortify vegetables involves many factors that must be addressed before developing a crop to enhance a specific component. Until absorption, the micronutrient that has been fixed in the soil should be made accessible to the plant. Plant cells have a variety of transporter systems that help them absorb minerals from the soil (Ram *et al.*, 2019; Vishwakarma *et al.*, 2019). A genetic technique should be used to improve the productivity of these mineral uptakes (Zhu *et al.*, 2013). Micronutrient redistribution within the plant system is the second target. The source-sink relationship can aid in nutrient maintenance in the plant system. Foliar application of micronutrients like Zn can boost accumulation in the shoot, but Zn transport in the phloem limits accumulation in fruit, seeds, and tubers (White and Broadley, 2011).

Biofortification Using Transgenic Approaches:

Tomato

Antioxidants: Antioxidants found in fruits and vegetables include anthocyanins, carotenoids such as lycopene and ß-carotene, as well as vitamins C and E. There are higher levels of glutathione and ascorbate, the soluble antioxidants of primary metabolism, as well as total antioxidant activity, in transgenic fruit that accumulate trans resveratrol (Giovinazzo, 2005).

Carotenoids -rich tomato: Lycopene is a powerful antioxidant that has been shown to reduce the risk of epithelial cancer and improve human health. As a result, there is a possible interest in genetically manipulating tomato carotenoids levels in order to improve the nutritional quality of the tomato crop. By generating phytoene from GGPP, the Psy-1 enzyme acts as a catalyst for the first step of the carotenoid biosynthesis pathway (geranylgeranyl diphosphate). The Psy-1 gene was expressed continuously in tomato to increase the carotenoid content of the fruit (Bergougnoux, 2014).

Anthocyanin-rich tomato: *Arka Vikas* was developed to increase the anthocyanin content in the fruit of a profitable tomato cultivar by Agrobacterium-mediated transformation of two transcription factors Del and Ros1. The transgenic fruit had an average anthocyanin content of 0.1 mg g-1 fresh weight, which was 70-100 times higher than the control fruit (Maligeppagol, 2013).

Flavanols rich tomato: Tomato transformed with the chalcone isomerase-encoding Petunia chi-a gene. The resultant transgenic tomato lines had a 78-fold increase in fruit peel flavanols, owing to an accumulation of rutin. Ectopic expression of chalcone isomerase, a single biosynthetic enzyme, resulted in a 78-fold increase in total fruit flavanols (Verhoeyen, 2002).

Folate -rich tomato: The folate content of food plants and how increasing PABA levels in grains can help Engineering a moderate increase in pteridine production could dramatically improve agriculture and rural development: spatial issues, challenges, and approaches 91. When vine-ripened tomato fruit was crossed with transgenic PABA- and pteridine overproduction traits, it accumulated up to 25 times more folate than the control (Garza, 2007). To increase levels of the polyamines spermine and spermidine in tomato fruit during ripening, researchers used a yeast S-adenosylmethionine decarboxylase gene (ySAMdc; Spe2) fused with a ripening-inducible E8 promoter. This resulted in longer vine life, better fruit juice consistency, and more lycopene (Mehta, 2002).

Potato: Overexpression of chalcone isomerase (CHI), dihydroflavonol reductase (DFR), and chalcone synthase (CHS) genes resulted in a substantial increase in measured anthocyanins and phenolic acids in potatoes (Lukaszewicz Marcin, 2004).

Starch-rich potato: In potato tubers, starch is the primary carbohydrate storage portion, accounting for up to 70% of the tuber's dry matter. When the bacterial ADPGP Pase gene from Bacterium Escherichia coli was transferred to potato, the transgenic plant developed tubers with a high starch content (Stark, 1992).

Protein-rich potato: Protein-rich potato expressing the seed protein gene AmA1 will increase nutritive value by expressing a non-allergenic seed albumin gene from Amaranthus hypochondriacs (Amaranth Albumin 1). On a biochemical level, AmA1 expression in both types of transgenics results in a significant increase in all essential amino acids, especially lysine, tyrosine, and sulphur amino acids, as well as an increase in total protein content. Through hybridization and selective breeding methods, a project on Bio Cassava Plus was developed to increase vitamins A and E, minerals zinc and iron, decrease cyanogen content, protein content, delay postharvest deterioration, and produce virus-resistant varieties (Chakraborty, 2000).

Amino acids-rich potato: Transfer of the high essential amino acid encoding heaae gene to potato clones K-2 and K-7 resulted in an increase in essential amino acids. These 292 base pair synthetic gene fragment (HEAAE-DNA) codes for a protein that contains around 80% essential amino acids (Yang, 1989).

ß-carotene-rich potato: The overexpression or simultaneous expression of genes encoding chalcone synthase (CHS), dihydroflavonol reductase (DFR), and chalcone isomerase (CHI) resulted in an increase in measured phenolic acids and anthocyanin. In the most affected transgenic line, the crtB gene was also transformed into Solanum phureja (cv. Mayan Gold), resulting in an increase in total carotenoid content to 78 g carotenoid g-1 DW. Transgenic potato plants expressing an Erwinia uredovora crtB gene encoding phytoene synthase, specifically in the tuber of Solanum tuberosum. cerevisiae, have been developed to increase the carotenoid content of potato tubers. Carotenoid levels reached 35 g carotenoid g-1 DW in developing tubers of transgenic crtB Desiree lines, and the balance of carotenoids changed dramatically compared to controls. The crtB gene was also inserted into Solanum phureja (cv. Mayan Gold), which resulted in an increase in total carotenoid levels. Spatial Issues, Challenges, and Approaches in Agriculture and Rural Development In the most affected transgenic line, the carotenoid content dropped from 92 to 78 g carotenoid g-1 DW. Lutein, antheraxanthin, ß-carotene, and violaxanthin were the most abundant carotenoids in these tubers (Ducreux, 2005).

Cauliflower: Cauliflower cloning is a success. Manipulation of chromoplast formation to provide an important metabolic sink for carotenoid sequestration and deposition has a significant impact on carotenoid accumulation, according to or gene. The use of the or gene to increase carotenoid content in transgenic potatoes demonstrates a novel approach to complementing effects based on the expression of carotenogenic genes to increase carotenoid levels in food crops (Zhou, 2008).

Cabbage: High antioxidant properties and anthocyanin content in red cabbage can reduce the risk of cancer, cardiovascular disease, and brain disorders (Draghici, 2013).

Carrot: Ca uptake can be boosted by genetically modified carrots with enhanced Ca levels, which may aid in the prevention of calcium deficiencies such as osteoporosis. The plant Ca transporter SCAX1 was expressed at higher levels in transgenically modified carrots (Park, 2003).

Pumpkin: According to the cooking methods used and the high content of total carotenoids in pumpkin, the total carotenoid and ß-carotene isomers content increased. Cassava is a root vegetable. Cassava without cyanogen. The cyanogenic glucoside linamarin is found in high concentrations in cassava. Many cassava cultivars have higher cyanogen levels in their roots (10-500 mg CN equivalents/kg dry weight) and leaves (200-1, 300 mg CN equivalents/kg dry weight) than the maximum Publication (Carvalho, 2014).

Conclusions:

The biofortification strategy increases everyone in the family's daily consumption of large amounts of nutrient-dense foods, particularly children and women who are most vulnerable to micronutrient malnutrition. Since food staples predominate in the diets of the poor, this policy indirectly targets economically disadvantaged households. Following a single investment in developing self-fortifying seeds, subsequent costs are minimal, and germplasm can be spread globally. This part of the plant breeding program's multiplication through distance and time makes it cost-effective. Biofortification is a simple way to target malnourished people in rural areas, as it offers naturally fortified foods to people who don't have access to commercially fortified foods, which are more readily accessible in towns. As a result, biofortification and industrial fortification are closely intertwined.

In the end, healthy nutrition requires a sufficient intake of a variety of nutrients and other compounds in levels and combinations that are still unknown. As a result, increasing the intake of a diverse range of non-staple foods in developing countries is an effective way to eradicate undernutrition as a public health problem. However, this will take decades to achieve, as will well-informed government policies and a sizable investment in agricultural research and other public and private infrastructure.

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