Biofortification of Vegetables

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Abstract

In the past few decades, the major concern on this planet was food security. After making a successful lead in food security now, the developing nations are focusing on nutritional security, which includes food that is enriched in minerals and vitamins. Micronutrients and vitamins are essential for human growth and development. Any deficiency of these components leads to "hidden hunger." Enhancing these components can alleviate malnutrition in women and children in the developing world. Micronutrients like Fe, Zn, Se, Mg, Ca, Iodine, and vitamins like provitamin A and folate are an important component of the biofortification program. Biofortification of vegetable with vitamins and micronutrients is the present need of an hour to fight different health issues faced by the developing countries. For biofortification of vegetable and other staple crops, three major techniques are used, viz. conventional breeding, agronomic approach (use of mineral fertilizer), and genetic engineering. These approaches have enormous potential to address this vitamin and micronutrient malnutrition. Many genes are available for the target traits by which it will be possible to improve micronutrient in vegetables. These tools can be very much helpful in improving the level of micronutrients and vitamins by several-fold in staple cereals and vegetables.

Keywords

Biofortification · Vegetable · Iron · Zinc · Iodine · Selenium · Provitamin A

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5.1 Introduction

Agricultural research in developing countries is mainly focusing on increasing cereals production and productivity. This scenario is due to as cereal is a cheaper source of food for the poor population, and along with carbohydrates, it also provides protein, lipid, micronutrients, vitamins, phenols, antioxidants, which have huge health benefits. But most importantly, the challenges may be come across by the year 2050 when the world population will exceed 9 billion, and to feed this population, huge pressure will be on agriculture (Hubert et al. 2010). The modern technologies used in agriculture and allied fields are successful in fulfilling the demand for food and feed of poor populations in developing countries. In the last few decades, research and development in developing countries focused mainly on the production of food rather on the nutritional aspects. But now the time has come where the food production along with better nutritional quality to be taken care (Bechoff et al. 2009). The production of food and feed must be increased many folds, while on the other hand, this has to be done by preserving the agroecosystem so that the balance has to be maintained. Micronutrient undernourishment is a major issue for more than half of the world's population, which is prominent in developing countries (Ortiz-Monasterio et al. 2007). The primary source of vitamins and minerals in developing countries mainly depends upon staple food. But staple foods like rice, wheat, and maize are rich in carbohydrate but deficient in vitamins and minerals. The human need to consume a relatively small amount of macroelements, a trace amount of microelement (Fe, Cu, Zn, I, and Se) and vitamins along with a large amount of carbohydrate, protein, and lipids (Welch 2002) to maintain a healthy lifestyle.

The edible part of plants like grain or seed can be widely used to prepare foods which contain a low level of micronutrients and have low bioavailability to human. This needs to be improved, and uptake efficiency should be increased in order to get more bioavailable micronutrients and vitamins. But more than half of the world's population suffers from micronutrient undernourishment as most of the staple food which is consumed in the developing countries are low in vitamins and minerals (Ortiz-Monasterio et al. 2007). Thus, biofortification is an essential requirement for the enhancement of these vitamins and minerals may be through conventional breeding or by genetic engineering approach.

After World War II, agricultural research is diverted towards cereals production and productivity. But this has to be a shift from cereals to vegetables where there is ample scope to increase the nutrition quality and reduce the hidden hunger. Kennedy et al. (2003) reported that the out of three-person in one person suffers from hidden hunger due to lack of minerals and vitamins in their diet, which ultimately leads to serious health consequences. Agricultural crops are the primary source of micronutrients and vitamins, which are essential for human growth and development. In the developing countries, the women, infants, and children of low-income families mostly suffer from iron, zinc, and vitamin A deficiencies, which account for over three billion people (Welch 2005). These in micronutrient deficiencies result in increased morbidity, and mortality rates, slow development of the nation, and permanent impairment of cognitive development in children and infants as all categories of people cannot afford the high quality and costly fruits and vegetables for gaining nutrition. Thus, an agricultural-based approach has been proposed as supplementary strategies for the breeding of staple food crops for higher vitamins and micronutrients. This method of increasing the vitamins and minerals by breeding approach or by genetic engineering has been termed as biofortification (Nestel et al. 2006). Thus, biofortified staple cereals and vegetables will be beneficial for the sparse population of the world and will help to increase nutritional status. This will lead to a significant advantage in health and economic benefits.

The important concern for biofortification is that after the development of variety, there should be widespread adoption by the farmer. The crop has to reach the needy poor people. Vegetables, fruits, dairy, and meat products are rich in vitamins and micronutrients, but they are expensive for poor people. They rely on few starchy staples (rice, wheat, maize, and potato); as a result, the intake of dietary diversity becomes a luxury, and poor people cannot afford it (Gómez et al. 2013). The extent of diseases due to malnutrition and mineral deficiencies is so high that the World Bank estimated the combined economic cost of mineral deficiency in developing countries and could waste as much as 5% of its gross domestic product (GDP). The deficiency of micronutrient and vitamins has a significant impact and burden on society which ultimately leads to an increase in susceptibility to infectious diseases, physical impairment, cognitive losses, blindness, and premature mortality. Comprehensively, the deficiency of provitamin A, Fe, I, Zn, and Se is reported to have a maximum percentage of disease burden, negative impact on the public (Black et al. 2008; Stein 2010).

5.2 What Is Biofortification

Biofortification can be defined as the development of micronutrient-dense staple crops (cereals and vegetables) using traditional plant breeding practices, modern biotechnology, and agronomical approached. In this process, the concentration of plant-derived nutrition and vitamins is increased in the edible organ during the growth and development of the plant. (O'Hare 2015). This is the process of breeding nutrients in the food crops and provides a low cost, sustainable, and long term delivery of adequate micronutrients. It is a technique where the edible parts like grain, straw, roots, fruits, and tubers are enriched with micronutrients and vitamins through the appropriate breeding method and biotechnological tools (Bouis 2000; Saltzman et al. 2013). Biofortified staple food may not contain a high level of essential vitamins and micronutrients as compared to industrially fortified foods, but they can help to reduce "hidden hunger" by increasing the daily adequacy of micronutrients uptake by the individual throughout the life cycle (Bouis et al. 2011). The strategies of biofortification involve agronomic approach, conventional breeding, and genetic engineering approach (Fig. 5.1). Moreover, biofortification provides a possible means of disseminating the technology and food to a malnourished population where there may have limited access to diverse kinds of diet,

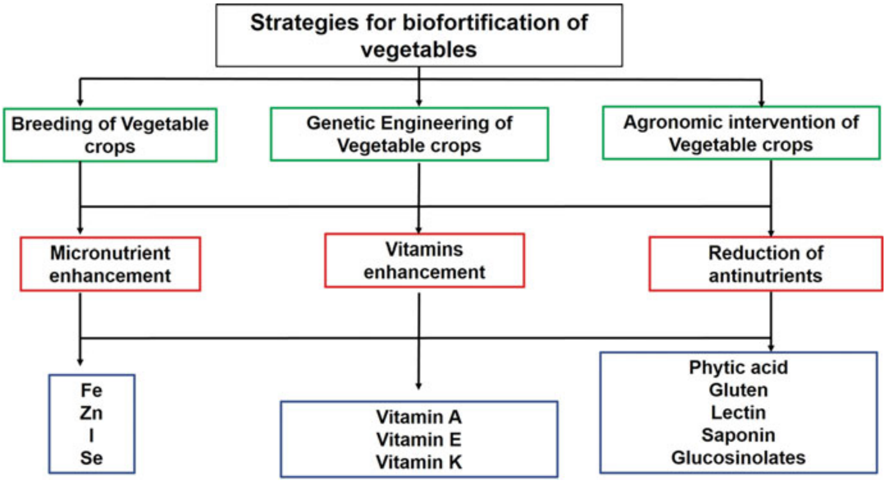

Fig. 5.1 Strategies for biofortification of vegetables with the enhancement of mineral and vitamin, reduction of anti-nutrients to increase the bioavailability of micronutrients

supplements, and food, which are commercially fortified (Saltzman et al. 2013). Biofortification is the different types of fortification and can be significantly increasing the level of vitamins and nutrients in the living product (edible part), and its accumulation takes place by normal physiological process of the plant (O'Hare 2015).

The breeding of plants for enhancement of bioavailability of micronutrients in the targeted edible food is the low-cost approach for poor countries and their people who cannot afford high-quality food. Thus mitigation of micronutrient malnutrition can be done by this approach, and this will ensure better health (Waters and Sankaran 2011). The enhancement of micronutrients and vitamins in the staple food and vegetable will lead to more consumption of micronutrients, particularly in the poor people, which ultimately leads to a reduction of malnutrition (Das et al. 2017).

Increasing phytonutrients and vitamins in a plant with a lesser life cycle which have short juvenile period to reach the flowering stage like in vegetable can be easily achieved. But this will be difficult for the tree-fruit and nuts where the juvenile period is much longer (O'Hare 2015). The principal objective of biofortification in vegetables should be the production of the nutritionally enriched vegetable crop so that the negative economic and health consequences can be overcome by managing mineral and vitamin deficiencies in humans. Among crops cultivated the fruits and vegetables have a rich source of genetic diversity for micronutrients, and hence they can be easily biofortified through conventional breeding, marker-assisted breeding, or genetic engineering (Das et al. 2017).

5.3 Importance of Micronutrients and Vitamins

Micronutrients and vitamins are an essential component in the growth and development of plants and animals. It is essential for metabolism like in redox reaction and also acts as the cofactor in various biochemical reactions inside the cell. Humans hardly synthesize vitamins, and the major vitamins are taken by plant or animal sources (Grivetti and Ogle 2000; Asensi-Fabado and Munné-Bosch 2010). Vitamins found in fruits and vegetables have strong antioxidant potential, which includes vitamin A, E, and K (lipid-soluble); vitamin B and C (water-soluble). These vitamins are synthesized and stored in the plant part, which are edible like in grain if seeds are used to prepare food. Application of breeding method and genetic engineering for biofortification with various micronutrient (Fe, Zn, I, and Se) and vitamins (vitamin A, B, C, E, and K) is relatively cost-effective and efficient method for counteracting deficiency for human and farm animals (Lyons et al. 2004; Nestel et al. 2006; Zhao and McGrath 2009). It was estimated over 60% of the world's population is deficient in Fe, 30% are deficient in Zn as well as in iodine, and 15% are Se deficient (White and Broadley 2009). Fruits and vegetables are rich in natural vitamins and minerals, which can act as an antioxidant that limits the cell damage from free radicals, which are produced during stress. There are studies related to cancer, where it was found that eating fruits and vegetables can prevent various types of cancer (Wargovich 2000; Tuama and Mohammed 2019). Thus for a healthy lifestyle, the consumption of fruits and vegetables should be done, and a balanced and complete diet should be maintained.

To meet metabolic need human requires at least 49 nutrients, out of which 20 minerals elements are essential for human health. There are seven major elements (Ca, P, K, S, Na, Cl, and Mg) and 12 trace elements (Fe, I, Cu, Zn, Mn, Co, Cr, Se, Mo, F, Sn, Si, and V). All the minerals are needed to supplemented or taken from other sources such as plant or animal products. During the early 1960s, the green revolution made the earth capable of fighting against food security (Pinstrup-Andersen and Hazell 1985). But this reduced the local production of fruits, vegetables, and legumes, which is the primary source of micronutrients in the people (Welch and Graham 2004). Fruits and vegetables are the primary source of mineral and vitamins along with dietary fiber that helps to improve the digestive function and lower the risk of type II diabetes, obesity, and related disorder.

5.3.1 Vitamins

Fruits and vegetables play an important role in human health as they contain important essential components like nutrients, vitamins, and phytochemicals, which may reduce the risk of chronic diseases. Due to improper nutrition, the present lifestyle and unhealthy diet lead to the development of serious diseases like diabetes, obesity, certain types of cancer, stroke, inflammation, and other cardiovascular diseases (Cömert et al. 2019). Vitamin is an essential component of human health, growth, and development. As discussed earlier, vitamins cannot be produced by humans but need to be consumed from another source like plants and animals.

It was estimated that about 250 million pre-school children are under the risk category of provitamin A deficiency along with the significant proportion of pregnant women who are at risk are suffering from provitamin A deficiency. Due to this provitamin deficiency, the children are at higher risk. It causes visual impairment, blindness, and an increase in the risk of illness from diseases like diarrhea and measles. (WHO 2016). It was estimated that in developing countries, the death of 5–10 million children takes place every year due to this above issue (Anderson 2019). Provitamin A is derived from a plant from the carotenoid pathway. It is a derivative of carotenoid, which includes retinol and its ester, retinal, and retinoic acid. This vitamin plays an important role in vision, immune response, growth of the epithelial cell, bone growth, and regulation of adult genes (Tumuhimbise et al. 2013). Moreover, childhood blindness is due to vitamin A deficiency due to which around 250,000–5,00,000 children go blind, and it is more prevalent in developing countries like sub-Saharan Africa and Southeast Asia (Nestel et al. 2006).

The primary source of provitamin A or carotenoid is commonly found in animal products like egg and milk. The precursor of vitamin A or β-carotene is abundantly found in the colored fruits and leafy vegetables. Change in β-carotene or provitamin A content was studied in dried sweet potatoes when it is processed. It was reported by Bechoff et al. (2009) that *trans-β*-carotene was retained significantly in hot air cross-flow as compared to sun drying. Drying of sweet potato slices was done in an air oven for 12 h at 60 °C; it was found that about 30% reduction in total carotenoid was observed (Hagenimana et al. 1999). Lower retention of provitamin A was reported in artificial drying as compared to natural sun drying. Thus, provitamin A or trans-β-carotene decreases after processing of sweet potato (Hagenimana et al. 1999; Van Hal 2000; Kosambo 2004).

Provitamin A or carotenoid is a powerful antioxidant which prevents the free radical that damage the cell membrane, which are produced during stress. It is used to build new cells and is critical for healthy brain development and nerve function, which ultimately slows down the aging process in humans. It also promotes the growth of strong teeth and bones. The formation of rods and cones in the retina of the eye is dependent on vitamin A which helps us to improve visual (Mata et al. 2002).

5.3.2 Iron (Fe)

Iron (Fe) is an essential micronutrient for humans, animals, and plants. It is redox-active metal, which catalyzes the oxidation-reduction reaction and regulation of cell growth and differentiation. It is an integral part of many proteins and enzymes where it acts as a cofactor for physiological function. It also plays a vital role in metabolic pathways like the electron transport chain of respiration in mitochondria. Most importantly, the human body’s Fe is contained in red blood cells (Gómez-Galera et al. 2010; Jomova and Valko 2011). If Fe deficiency occurs in the body, the amount of hemoglobin is also affected, and the symptoms of anemia take place along with tiredness, weakness, and inability to concentrate (Das et al. 2017). Deficiency of Fe during childhood and puberty stage may impair physical growth, mental development, and poor learning capacity. Moreover, severe anemia increases the risk of dying during childbirth (Beard 1994). It also catalyzes the formation of provitamin A from carotene and also induces antibodies synthesis, which ultimately enhances immunity (Semba 1994). The RDA (mg Fe day^{-1}) for Fe is 10, 8, and 18 for children, adult males, and adult females (Trumbo et al. 2001).

Fe is derived from animal products like milk and meat and also from plant products. Before absorption of Fe in body, the bound Fe should be well hydrolyzed or solubilized. But the absorption of Fe largely depends upon the presence of enhancer and inhibitory substances (Baltussen et al. 2004). The compounds like ascorbic acid and cysteine enhance Fe absorption in the gut, whereas compounds like phytic acid chelate with Fe and Zn which inhibit the absorption of Fe in the gut (Kumar et al. 2017).

5.3.3 Iodine

Iodine is a crucial microelement which plays an essential role in the metabolism of human. Its deficiency can lead to a severe disorder of thyroid and the inadequate synthesis of hormone which are released from thyroid. The RDA of iodine is 40–200 μg day^{-1} (Dai et al. 2004). It has multiple roles in human growth and development, which involve enhancement of protein, regulating the energy transfer, and maintaining the central nervous system (Gerber et al. 1999). It controls the production of thyroid hormone known as thyroxine (T4) and triiodothyronine, which is the major hormone responsible for maintaining the above function. The reports from WHO exposed that about 1.6 billion people are suffering from iodine deficiency throughout the world (WHO 2016).

The important primary source of iodine supplementation followed nowadays is seafood and iodized salt. The consumption of seafood is very much helpful to obtain iodine supplementation, but it does not meet the need of iodine-deficient area and the vegetarian population. Moreover, the supplementation of food products with iodine may be helpful in controlling the deficiency of iodine, but it may be difficult to control the losses during transport, storage, and food cooking (Winger et al. 2008). The 80% of the requirement of iodine in the human body comes from the edible vegetable, which is grown under the natural condition, and about 99% bioavailable iodine can be in this food (Welch and Graham 2004).

5.3.4 Zinc

Zinc (Zn) is an important trace microelement for microorganisms, plant as well as animal (Broadley et al. 2007; Prasad 2008). It is the cofactor for various enzymes and proteins. It is involved in different physiological functions, such as the functioning of the immune system, protein synthesis, DNA synthesis, wound healing, and cell division (Bao et al. 2010). A large number of enzymes and proteins are dependent upon Zn to maintain their structural stability and transcription factor. It also helps to improve and maintain the immune system, thus prevent infection and diseases (Prasad 2008; Bao et al. 2010). Moreover, Zn is required to activate over 300 enzymes in the human system. In humans and animals, it plays an important role in the functioning of reproductive health, sensory function, digestive system, and neurobehavioral development (Levenson and Morris 2011). The RDA (mg Zn day^{-1}) for children is 5, for adult males is 11, and 8 for adult females (Trumbo et al. 2001).

It also participates in the synthesis and degradation of carbohydrates in plants. Along with carbohydrates, it also involves the synthesis of lipid, protein, and nucleic acid (Brown et al. 1993). It is the only element that is involved in all the six classes of enzymes, namely oxidoreductase, transferase, hydrolases, lysases, isomerases, and ligases (Barak and Helmke 1993). It occurs in the plant as a free ion and may be complexed with low molecular weight compounds. The deficiency of Zn in plants leads to inhibition of photosynthesis, decreases the production of auxin hormone (as the cofactor for the synthesis of auxin), and decreased rate of respiration. Moreover, its deficiency in the plant may also lead to an increase in the disruption of the plasma membrane as many antioxidant enzymes may be inhibited. This leads to the enhancement of reactive oxygen species in the cell, thus damaging the cellular compartment and organelles (Brown et al. 1993). Fe can have a negative effect on Zn absorption in the human gut if given together in a supplement. Most importantly, it was observed that protein meals enhance Zn absorption. Other compounds such as amino acid and low molecular weight ions (EDTA and organic acid like citrate) are shown to have a positive effect on Zn absorption and have been used for Zn supplement (Zhao et al. 2012). The bioavailability of Zn in cereals, legumes, vegetables, nuts, and wholegrain can be inhibited by phytic acid. Phytic acid inhibits the release of Zn from the food by chelating it and thus making it unavailable for absorption in the human gut (Kumar et al. 2017).

5.3.5 Selenium

Selenium (Se) is a trace element which is required by human and animal in very minute quantity, but it is essential for human growth and development. However, in higher concentrations, it has a toxic effect. The protein which contains Se is known as selenoprotein, which has a structural and enzymatic role. It also acts as antioxidant element to prevent from the damage of the free radical formation. The other property of Se also includes the catalysis of active thyroid hormone. It also prevents immune response disease and helps to maintain the immune system, which further acts as a key nutrient in counteracting the development of virulence and inhibiting HIV progression to AIDS (Rayman 2000). The deficiency of Se in the blood can lead to various health implications like cancer and cardiovascular diseases (Brown and Arthur 2001). It was reported that its deficiency might lead to adverse mood states, oxidative stress, and inflammation. The higher intake of Se is associated with the reduced risk of cancer (Rayman 2012). Maintaining Se intake in the diet may prevent the risk of lung, prostate, colorectal, and bladder cancers. The deficiency also leads to muscular dystrophy and muscle weakness.

The uptake of Se can take place in the plant as selenite, selenite, or organoselenium compounds. These compounds mainly include selenocysteine and selenomethionine, but they cannot take up as colloidal elemental Se of metal selenides (White et al. 2004). Se transported inside the plant cell through high-affinity sulfate transporter (HAST) in root cell (Terry et al. 2000; Li et al. 2008). Moreover, it is also transported through phosphate transporter (Broadley et al. 2006). The uptake of Se takes place in the roots where selenite is delivered to xylem and transported the shoot, and here, assimilation takes place where selenite is converted to organoselenium compounds (White and Broadley 2009).

5.4 Physiology of Biosynthesis of Vitamins and Micronutrients

Fortification of a food product is necessary for many countries, where there is a fortification with vitamin A in butter, margarine, and sugar, iodine fortification in salt, vitamins A fortification in mild, and folate fortification in cereals and legumes (Kumar et al. 2019). However, there were various drawbacks of fortification/supplementation. So the technology of biofortification came into action where enhancement of vitamin and mineral can be done in the edible part of staple food with the help of breeding or genetic engineering. So to understand the process of accumulation of vitamin and mineral in staple food, most importantly, the physiology of crop is to be explored. The basic pillars of the biofortification program are to understand the physiological process and bioavailability of nutrients and vitamins. So, the knowledge of the physiology of nutrient uptake, phytoavailability of micronutrients in the rhizosphere, and vitamin synthesis are essential (Bowen and Rovira 1991).

5.4.1 Vitamins

Vitamins are primarily synthesized in the plant. Out of which folates and provitamin A are mainly targeted for the biofortification program. Folates are the group of water-soluble vitamin B, which is also known as Vitamin B9, which consists of pteridine ring and a para-aminobenzoate moiety (*p*-ABA) and is linked with γ-linked tail with more L-glutamates (Strobbe and Van Der Straeten 2017). Folate biosynthesis takes place in the cytosol where the pterin branch is formed, and it yields 6-hydroxymethyl-dihydro pterin (HMDHP). Folates are present in mitochondria, plastids cytosol, and vacuoles (Chen et al. 1997).

The synthesis of carotenoid takes place de novo in the plant, and it is mainly involved in the synthesis of photosynthetic pigment and precursor for other molecules such as signaling molecules. It is synthesized in plastid and is an essential component of a healthy diet, antioxidant, and also a precursor of provitamin A (Giuliano 2017). The precursor of provitamin A is a C40 isoprenoid unit, which is a carotenoid. It is synthesized in plastid from the 2-C-methyl-d-erythritol 4-phosphate pathway. Geranylgeranyl pyrophosphate is formed after condensation of isopentenyl pyrophosphate and dimethylallyl pyrophosphate in the presence of isopentenyl diphosphate isomerase. Geranylgeranyl pyrophosphate is the precursor of other isoprenoid molecules, like gibberellins, quinones, provitamin A, and the isoprenoid moieties of chlorophylls and tocopherols which is the major constituents of chloroplast (Giuliano et al. 2000). The enzymes responsible for the synthesis of β-carotene are phytoene synthase (Psy), phytoene desaturase (Pds), ζ-carotene desaturase (Zds), and lycopene cyclase (Lcy) (Sandmann 2001).

5.4.2 Minerals

The uptake of micronutrients like Fe, Zn, I, and Se are generally taken place from the rhizosphere. These micronutrients are then transported from root to edible part of the plant. The uptake, transport, and accumulation of micronutrients are highly regulated and tightly controlled mechanism (Welch 1995).

5.4.3 Iron

The absorption of Fe takes place in the rhizosphere. The proton is released from plants in the rhizosphere, which lowers the pH of soil solution and thus increases the solubility of Fe^{3+} (Santi and Schmidt 2008). There are two strategies for the uptake of Fe in plant, viz. the strategy I and strategy II (Santi and Schmidt 2008). Strategy I takes place in all the plants except plant from Graminaceae family. The Fe uptake in this strategy takes place by reduction of ferric iron, and this is then bound to chelates (citrate and nicotianamine) and subsequent uptake of liberated ferrous iron. This step is mediated by an iron-regulated enzyme known as ferric oxidoreductase (FRO). The strategy II of uptake of Fe takes place in microorganism and grasses. In this strategy, there is a release of protein known as phytosiderophores in the rhizosphere. This protein takes up the ferric iron-loaded phytosiderophores-metal complex inside the plant through membrane-bound transporter (Robinson et al. 1999).

5.4.4 Zinc

The uptake of Zn primarily takes place by Zn transporter of the ZIP family, which is highly regulated in root and other tissues. The transcription factor which is responsible for Zn uptake in the plant is two bZIP families, and it is upregulated under Zn deficiency (Grotz et al. 1998). It is generally absorbed from soil as Zn^{2+} or $Zn(OH)_2$. Phytosiderophores have the same mechanism of uptake for Zn. Phytosiderophores like mutagenic acid, avenic acid, and nicotinamine are responsible for the uptake of Zn from the soil. Under deficiency condition, Zn chelates with phytosiderophores, and its uptake rate was found to be increased (Ueno et al. 2007). The mode of transportation of Zn between the cells can be symplastic or apoplastic. The factor which is responsible for absorption and desorption of Zn includes chemical form of Zn, total concentration of Zn in soil, pH of soil, organic matter content of soil, temperature of soil, carbonate and phosphate content of soil, microorganism content of soil, and other relative biological activities of plant (White and Broadley 2005).

5.4.5 Iodine

The soil contains a very low amount of iodine, and this amount is insufficient for humans and animals in comparison to their nutritional needs (Halka et al. 2019). It is present in a very trace amount which is fixed with organic matter, clay, and oxides of Fe and Al. The WHO recommended the daily dose of 5 g/day iodized salt (WHO 2013); however, the uptake of iodine is less. Iodine is taken by the plant in the form of iodate and iodite. The most prevalent form of iodine in soil solution is iodite (Fuge and Johnson 1986). The experiments on Chinese cabbage revealed that iodine uptake by this Chinese cabbage was more effective when iodine was supplied as iodate as compared to iodite in low concentration (Weng et al. 2008a). The iodine concentration in root was greater in as compared to leaf. The spray of iodine solution in cabbage and spinach increases the level of iodine in both roots and leaves (Weng et al. 2013). It was reported that the degree of phytotoxicity of iodine exists in soil solution, and iodide is more phytotoxic than iodate; this may be due to plants absorb a more reduced form of iodine (Weng et al. 2013).

5.4.6 Selenium

The micronutrient Se is not that essential for plant growth and development, but it is much more essential micronutrient for humans and animals (Fordyce 2013). In the plant, it enhances the antioxidant capacity, which helps to alleviate the heavy metal stress. The uptake of Se in the plant does not take in its colloidal elemental or selenide form, but in plant roots, the uptake takes place in the form of selenite, selenite and organoselenium compounds (White and Broadley 2009). The Se content in normal soil is around 0.01 and 2.0 mg Se kg^{-1}, whereas seleniferous soil contains Se concentration up to 1200 mg Se Kg^{-1} (White and Broadley 2009). It is mobile in the soil solution, and sometimes it strongly gets fixed with Fe and Al in soil (Broadley et al. 2006). It was reported by Hawkesford and Zhao 2007 that high-affinity sulfate transporter is involved in the transport of selenite across the plasma membrane of root cell, and phosphate transporter is involved in the transportation of selenite. Selenite is converted to organoselenium in root tissue and further transported all over in plant via xylem and redistributed in the plant in a similar manner as sulfur is distributed (Li et al. 2008).

5.4.7 Silicon

Recently silicon is getting wide attention due to numerous studies demonstrating beneficial role of the element for plant as well as human health (Deshmukh et al. 2017; Ratcliffe et al. 2017). Recently, International Plant Nutrition Institute (IPNI, http://www.ipni.net/) has announced silicon as beneficial element for the plant health. Similarly, numerous silicon based tonics and health supplements have been released worldwide (Scholey et al. 2018). In this regard, efforts are being made towards the enhancement of silicon uptake more particularly in vegetable crops (D'Imperio et al. 2016; Montesano et al. 2016). In a study performed by Montesano et al. (2016) performed Si biofortified in green bean pods by growing plants with the supplementation of Si-enriched nutrient solution. They have also showed effect of boiling and steaming cooking methods on Si content in the cooked green beans. Similarly, De Souza et al. (2019) study has shown that the silicon spray on leaves can promote biofortification and also help to increase the biomass and ascorbate content in Chard and Kale. However, most of the vegetables belong to Solanaceae and Brassicaceae family cannot uptake silicon from soil and such species are well known poor silicon accumulators (Deshmukh et al. 2015; Deshmukh and Bélanger 2016; Sonah et al. 2017). Efforts can be made through transgenic or genome editing approaches to make such species genetically capable to uptake and accumulate significant amount of silicon (Vats et al. 2019; Mushtaq et al. 2020).

5.5 Agronomic Biofortification of Vegetables

The agronomic biofortification of vegetables is one of the simplest and easy methods of biofortification. However, this strategy requires a long period and adequate funds, and this technique is useful in the countries where the genetic engineering method of biofortification is not well accepted. In this approach, generally, fertilizer is used either in the form of spray on leaves or the application of fertilizer in soil (Weng et al. 2008b). The biofortification of Fe and Zn was reported to be successful where the foliar application was used to enhance these nutrients in plant tissue and edible part (Saltzman et al. 2013). The agronomic approach for biofortification also includes management practices during the crop growing season. The package and practices like tillage, water management, and nutrient interaction are involved in enhancing micronutrient. Foliar application is the better option for agronomic biofortification, which requires less amount of Fe and Zn fertilizer as compared to soil application (Prasad et al. 2014).

5.6 Breeding of Vegetable for Biofortification of Vegetables

The current knowledge of all the processes for enhancement of micronutrients and vitamins in the edible part of the plant is very limited. More basic research is needed to accumulate the micronutrients, making micronutrients in bioavailable form and

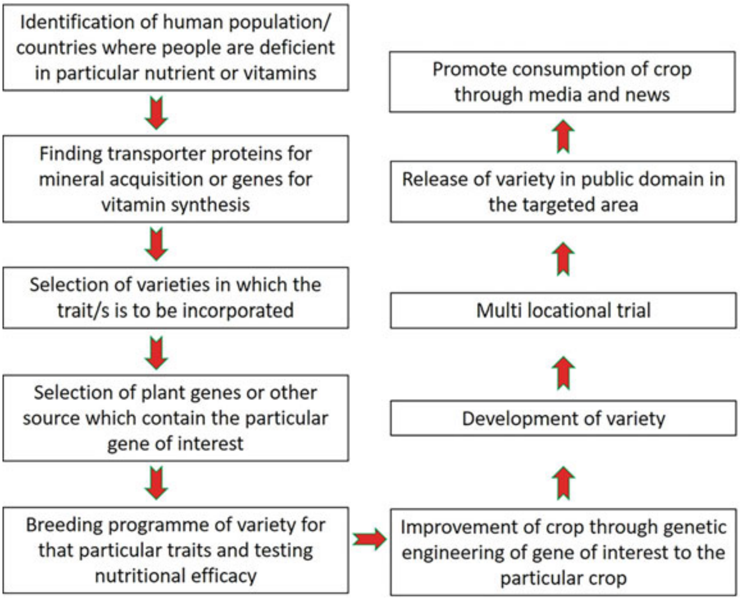

Fig. 5.2 Flow chart for biofortification of vegetable using a breeding program or genetic engineering as a tool

ultimate increasing vitamin in seed, grain, tuber, flesh, and other edible parts of staple cereals and vegetables. Using classical or modern breeding techniques and also with the help of genetic engineering, this can be possible to biofortify large amounts of crops and can be disseminated throughout the world (Welch and Graham 2004). The effort is for one time in the breeding of a biofortified crop. Once the plant is developed and is adopted by the farmer, then the seed can be reproduced, multiplied, and shared among all the farmer groups of the targeted area (Fig. 5.2). This will ultimately help to maintain high nutrient traits, overtime in that particular crop (Graham et al. 2001). The conventional breeding is based on natural variation and maybe a good alternative to genetic engineering experiment. It was found that the folate content in vegetables such as tomato and potato was found to be increased twofold in newly developed breeding lines (Hanson and Gregory 2011).

Due to the increase in the demand for the biofortified product, the production has to be increased using the genetic basis of plant breeding. The criteria have to be set for the breeding of vegetables for micronutrients and vitamins, which will meet the demand of farmers as well as the targeted people (Fig. 5.3). Firstly, vegetable production and productivity should be maintained so that it will be widely accepted by the farmer, and they must get the revenue as invested by them. The yield of biofortified vegetables must be more or at par with the previous version of that vegetable. Secondly, the micronutrient concentration in the vegetable should be achieved significantly so that it will have an impact on human health. Thirdly, the particular trait for the biofortification of vegetables should be stable between

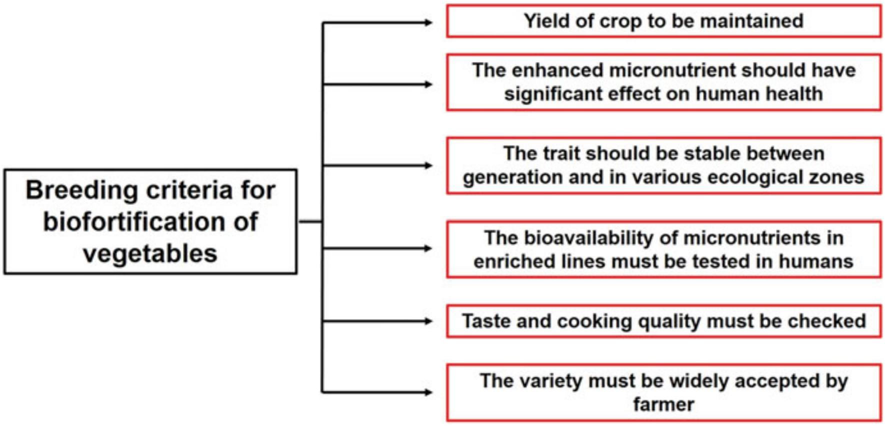

Fig. 5.3 Breeding criteria for biofortification of vegetables

generations. This is applicable to the various ecological and climatic zone where the vegetable is suitable for its growth. Fourthly, the micronutrient bioavailability should be maintained while cooking, and its level should not alter after cooking. Fifthly, the taste of biofortified products should be acceptable to the consumer. And the final criteria should be the acceptance by the farmer. More publicity and importance of crops should be disseminated to the farmer so that they will know the importance of growing of biofortified vegetables (Welch and Graham 2004).

The aim of CGIAR and HarvestPlus is to determine the genetic variability and heritability of the mineral trait and their stability across the different climatic and ecological zone, soil conditions, and a number of the gene responsible for its uptake and feasibility of breeding increased concentration of several minerals. The conventional breeding for more nutrition relies on the inheritance of genes responsible for the particular trait and favorable quantitative trait loci (QTL) from sexually compatible parental lines. Thus, biofortification by breeding has its own constraints of finding a natural variation of desired traits and the collection of vegetable germplasm, which is a time-consuming process Shimelis and Laing 2012. The recent advances in plant breeding techniques such as genome-wide association studies (GWAS) and marker-assisted breeding (MAB) is the powerful and important tools for biofortification. These newly advanced techniques helped breeder to identify the QTLs responsible for the increase in β-carotene and α-tocopherol up to 3.22 and 5.76 fold respectively (Azmach et al. 2013; Lipka et al. 2013).

A horticultural crop like cassava was targeted for enhancement of provitamin A. The variation in β-carotene concentration on cassava roots was screened from CIAT core collection (5500 genotypes). It was reported that after breeding for β-carotene content in cassava its content ranged from 0.1 ± 2.4 mg /100 g (Chávez et al. 2000). It has been suggested that the biofortification breeding programme for Se and iodine should be done simultaneously and the primary target for both the nutrient is thyroid and its metabolism (Lyons et al. 2005). Along with the breeding strategies, some research intervenes the agronomic practices to get a better result. Weng et al. (2008b) applied iodine fertilizer along with diatomaceous earth to radish (*Raphanus sativus* L.), spinach (*Spinacia oleracea* L.), and Chinese cabbage (*Brassica chinensis* L.) and found that iodine concentration was high in the leaves of these vegetables. In another experiment, Zhu et al. 2003 reported that iodate ion had a less detrimental effect on biomass production of spinach as compared to iodide ion (Zhu et al. 2003). Landini et al. (2011) study the uptake of iodine in tomato and its concentration in its fruit using radioactive iodine. They concluded that the iodine concentration was higher when plants were supplied with iodine hydroponically. Greenhouse experiment on soil-grown spinach revealed that the addition of iodate and iodide in the soil does not lead to an increase in biomass of spinach (*Spinacia oleracea* L.), but there was an increase in iodine concentration in leaf tissues. However, iodate is accumulated in the leaf tissues as compared to iodide (Dai et al. 2006). To understand the genetic variability of micronutrient content in potato germplasm breeding program was carried out in eight clones of potatoes. After analysis for Cu, Fe, Mn, and Zn content in potato clones, it was found that the difference between the clones was significant for micronutrient (Haynes et al. 2012). These variations in potato germplasm for micronutrients are large and can be used to improve the quality of the potato through a breeding program.

The identification of the genetic variability of the crop should be screened in which there is more accumulation of targeted micronutrients. The screening process should be emphasized on the rate and proportion of accumulation of micronutrients in the edible part (Calderini and Ortiz-Monasterio 2003). This is a one-time process, and once the high-yielding, high vitamins, and nutrient lines are developed, this has to be tested in multilocation for confirming its stability to grow in region-specific (Fig. 5.2). Conventional plant breeding is a cost-effective method, which is a widely accepted method for making plant biofortified and stable. The sustainable and cost-effective solution may be provided by plant breeding, and this will help to deliver micronutrients and vitamins to the targeted population. However, the uptake and accumulation of micronutrients in crops such as vegetables are regulated by polygene and having minor effects. Therefore, the conventional breeding of biofortification approaches has met with only marginal success (Naqvi et al. 2009). But in the absence of adequate genetic variability and variation among traits gene effect, genetic engineering will be more viable for the enhancement of micronutrients at the desired level. Despite various techniques in conventional breeding such as heterosis, transgressive segregants, mutational breeding, quantitative genetics, marker-assisted breeding, QTL mapping, etc. to explore the genetic variability for vitamins and micronutrients, it takes more time and labor as compared to genetic engineering. With the powerful tools such as “omics” technologies, gene editing tools like transcription activator-like effector nucleases (TALENs) and CRISPR/Cas9 help to increase the opportunity for new biofortification strategy in very less period of time.

There are some other examples of successful biofortification programs where the products are disseminated to the public. The Cowpea varieties Pant Lobia-1 and Pant Lobia-2 were released by G.B. Pant University of Agriculture and Technology, Pantnagar, India which was biofortified with high Fe and Zn respectively. Pant Lobia-1 and Pant Lobia-2 were released by Uttarakhand Government, India in 2008 and 2010, respectively.

5.7 Biotechnological Tools for Biofortification of Vegetables

Biotechnology is a powerful tool for the biofortification tool, which is being used worldwide to combat the seriousness of mineral and vitamin deficiency. The recent advancement in the tools and techniques of genetic engineering enables to incorporate the trait which cannot be possible through conventional breeding (Chaudhary et al. 2019; Rana et al. 2019). Not only one trait, but multiple traits or pathway can be targeted using this technology. The classic example is of increasing the bioavailability of micronutrients, enhancing β-carotene, ascorbate, and folate in a single plant (multivitamin corn) (Carvalho and Vasconcelos 2013).

The goal for biofortification of vegetable by genetic engineering includes several points which need to be considered before designing the crop for enhancing particular component (Fig. 5.4). The micronutrient which is fixed in soil should be made available for the plant before absorption. Various transporter systems present in plant cells involved in the uptake of mineral from soil (Ram et al. 2019; Vishwakarma et al. 2019). The uptake efficiency of these mineral uptakes should be increased using a genetic tool (Zhu et al. 2013; Pinto and Ferreira 2015). The second goal is the redistribution of micronutrients within the plant system. The source and sink relationship will help to maintain the nutrient in the plant system. The accumulation of micronutrients like Zn can be enhanced in the shoot by foliar application, but accumulation in fruit, seeds, and tubers is limited by Zn transport in the phloem (White and Broadley 2011). Thirdly, using the genetic tool, the pathway is

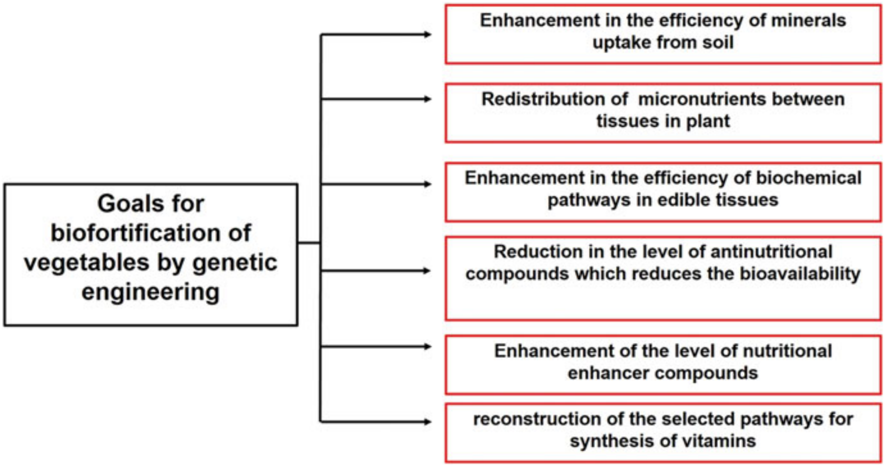

Fig. 5.4 Goals for biofortification of vegetable by a genetic engineering approach

responsible for enhancing the efficiency of the biochemical pathway in edible tissues. Fourthly, the biotechnological tool can be helpful in reducing the anti-nutritional compound, which ultimately affects the bioavailability of micronutrients. Fifthly, the level of nutritional enhancer can be increased by the overexpression of gene, which is responsible for nutrient bioavailability (Rawat et al. 2013). Lastly, the modification in the pathway leads to the production of higher vitamin in the edible part of the plant and consumption of which can reduce the incidence of malnutrition.

The studies on the bioaccessibility and bioavailability of β-carotene from plant matrixes revealed that the transgenic crops such as cassava, sweet potato, melon, sorghum, and potato perform better in terms of bioaccessibility and bioavailability as compared to non-transgenic plant (Failla et al. 2009, 2012; Thakkar et al. 2009; Fleshman et al. 2011; Lipkie et al. 2013). The major factor that affects the bioaccessibility and bioavailability is processing and cooking. The pathway of bacterial origin was incorporated in potato where there is a synthesis of β-carotene (provitamin A) from geranylgeranyl diphosphate in the MEP pathway (Diretto et al. 2007). They used a tuber-specific or constitutive promoter to express three gene encoding phytoene synthase (CrtB), phytoene desaturase (CrtI), and lycopene beta-cyclase (CrtY) from *Erwinia* in potato. It was reported that the carotenoid content and β-carotene content was enhanced 20 and 3600-fold respectively in transgenic potatoes (Diretto et al. 2007). The other technique of making a transgenic plant is by blocking the rate-limiting enzyme in the pathway. Römer et al. (2002) developed transgenic potato, which was biofortified with zeaxanthin and β-carotene by silencing the ZEP gene. The transgenic line was shown to increase zeaxanthin content up to 130-fold in potato tuber. Tomato is rich in lycopene and β-carotene, 5–15% depending upon the varieties and genotype. The transgenic approach was made by overexpression of phytoene synthase and phytoene desaturase using *35S::tp::crtI* promoter. The leaves of *crtI* tomatoes, β-cyclic carotenoids were enriched (Giuliano et al. 2000). Metabolic engineering efforts that overexpressed two folate synthesis genes in combination have increased folate levels by up to 25-fold in tomato fruit and 100-fold in rice grains (Hanson and Gregory 2011).

The overexpression of gene FEA1 from *Chlamydomonas reinhardtii* in cassava and sweet potato leads to successful enhancement of Fe accumulation in edible tuber tissue (Chávez et al. 2007). Cation transporter families such as ZIP (ZRT, IRT-related protein) and CDF (Cation diffusion facilitator) play an important role in the Zn uptake and translocation in the plant. IRT2 protein of the ZIP family was identified in Arabidopsis thaliana root cell, which significantly contributes to Zn uptake (Korshunova et al. 1999). The studies were carried out to enhance Fe content using a transgenic approach. Lactoferrin is a Fe-chelating glycoprotein from human milk and is a family of transferrin family, and ferritin is the protein that can store 4500 Fe in the bioavailable form (Kanyshkova et al. 2001). The transgenic plant was developed using the rice glutelin-1 promoter to increase the Fe content and was found that Fe content was increased significantly as compared to control (Nandi et al. 2002). The overexpression of the gene encoding Fe (III) reductases that enhance Fe uptake gene in the non-graminaceous plant is one of the strategies to biofortify the plant (Connolly et al. 2003).

Anti-nutrients such as phytic acid and tannins inhibit the absorption of Fe, Zn, and Ca in human and animal gut, thus reducing the bioavailability of micronutrients (Welch and Graham 2004). There is intra-specific variation in the phytate content in the edible portion of cereal grain and is independent of Fe and Zn concentration. However, Fe and Zn are inhibited by phytic acid content, and the bioavailability of Fe and Zn depends on cooking and processing (Kumar et al. 2017). By reducing phytic acid in cereals and vegetables, the bioavailability of Fe and Zn can be increased. It was reported that by knocking down enzymes of the IP6 pathway, the mineral bioavailability was enhanced. Moreover, the overexpression of phytase and phytate-degrading enzyme in the edible portion can also decrease the level of phytic acid, which ultimately enhance micronutrient bioavailability (Goto and Yoshihara 2001).

Iodine is an essential component of the hormones produced from the thyroid gland in humans, and it plays many vital roles with respect to the growth and development process (Velasco et al. 2018). It was reported in tomato that the expression of a gene such as HMT (encode for methyltransferase), SAMT (encode for salicylic acid carboxyl methyltransferase), and S3H (encode for salicylic acid 3-hydroxylase) could enhance iodine concentration in tomato fruit. In potato, the content of Se was increased under tropical climate by application of Se in small doses. The concentration of Ca, along with Se, was reported to be increased (de Oliveira et al. 2019).

5.8 Future Thrust

The current researches are now focusing on maintaining a healthy life by consumption of nutraceutical supplements as well as healthier diet from fruits and vegetable. So, there is scope to enhance micronutrients and vitamins in vegetables on a large scale through biofortification program. This will help developing countries to overcome the issue of malnutrition or “the hidden hunger.” However, many breeding programs are focused on the improvement of production and productivity, tolerance to abiotic stress, resistance to biotic stress. But enhancing the quality of vegetables will help those developing countries to save revenue, which may be spent against the disease, which is caused by micronutrient and vitamin deficiency. To achieve biofortification of vegetable it requires the collaboration of plant breeder, plant physiologist, biochemist, molecular biologist and other nutrition scientists. However, the genetically modified crop may require regulatory approval from various committees before it is released. The recent advanced technology in the field of genetics and genome editing (TALENS, CRISPR/Cas9, etc.) will help this biofortification program to move at a greater pace. More particularly, use of CRISPR/Cas9 based genome editing is being widely used in crop plants including many vegetables (Vats et al. 2019; Mushtaq et al. 2020). The recent advancement in the genome editing provides numerous approaches to get desired genetic modification bypassing the regulatory issues associated with transgenic technologies.

5.9 Conclusion

The major area of research for developing countries after food security is nutritional security. Because the major population of the developing world is suffering from "hidden hunger" and combating this problem, the agricultural scientist is capable of changing the physiology of crops by biofortification of vegetables and cereals. There is much scope for plant breeders, molecular scientists, and genetic engineers to improve micronutrient density and vitamin content of staple food crops and vegetables for developing countries. Moreover, after the development of variety, which is rich in micronutrients and vitamins, it should be adopted by the farmer on a large scale without hindering its production and productivity. There is enough genetic diversity of vegetables available, and it has to be screened for a particular trait. For enhancing micronutrient in the plant, there should be a clear understanding of the mechanism of ion uptake from soil, redistribution within tissues, and homeostasis in the plant. Working on enhancing micronutrient and vitamin with the help of conventional breeding or by genetic engineering both requires particular traits that need to be incorporated. The recent advances in genetics made it possible to enhance micronutrient by reducing anti-nutrients such as reduction of phytic acid or tannins. Genome editing tools like ZFN, TALENS, CRISPR-Cas9, etc. have the potential to edit plant genes or knockdown undesirable traits and can be exploited for the biofortification of vegetables.

References

Anderson MD (2019) 6 comparing the effectiveness of structures for addressing hunger and food insecurity. In: Civil society and social movements in food system governance. Routledge, New York, p 124

Asensi-Fabado MA, Munné-Bosch S (2010) Vitamins in plants: occurrence, biosynthesis and antioxidant function. Trends Plant Sci 15:582–592

Azmach G, Gedil M, Menkir A, Spillane C (2013) Marker-trait association analysis of functional gene markers for provitamin A levels across diverse tropical yellow maize inbred lines. BMC Plant Biol 13:227

Baltussen R, Knai C, Sharan M (2004) Iron fortification and iron supplementation are cost-effective interventions to reduce iron deficiency in four subregions of the world. J Nutr 134:2678–2684

Bao B, Prasad AS, Beck FWJ et al (2010) Zinc decreases C-reactive protein, lipid peroxidation, and inflammatory cytokines in elderly subjects: a potential implication of zinc as an atheroprotective agent. Am J Clin Nutr 91:1634–1641

Barak P, Helmke PA (1993) The chemistry of zinc. In: Zinc in soils and plants. Springer, Dordrecht, pp 1–13

Beard JL (1994) Iron deficiency: assessment during pregnancy and its importance in pregnant adolescents. Am J Clin Nutr 59:502S–510S. https://doi.org/10.1093/ajcn/59.2.502S

Bechoff A, Dufour D, Dhuique-Mayer C et al (2009) Effect of hot air, solar and sun drying treatments on provitamin A retention in orange-fleshed sweetpotato. J Food Eng 92:164–171. https://doi.org/10.1016/j.jfoodeng.2008.10.034

Black RRE, Allen LHL, Bhutta ZZA et al (2008) Maternal and child undernutrition: global and regional exposures and health consequences. Lancet 371:243–260

Bouis HE (2000) Enrichment of food staples through plant breeding: a new strategy for fighting micronutrient malnutrition. Nutrition 16:701–704

Bouis HE, Hotz C, McClafferty B et al (2011) Biofortification: a new tool to reduce micronutrient malnutrition. Food Nutr Bull 32:S31. https://doi.org/10.1177/15648265110321s105

Bowen GD, Rovira AD (1991) The rhizosphere: the hidden half of the hidden half. In: Plant roots: the hidden half. Marcel Dekker, New York

Broadley MR, White PJ, Bryson RJ et al (2006) Biofortification of UK food crops with selenium. Proc Nutr Soc 65:169–181

Broadley MR, White PJ, Hammond JP et al (2007) Zinc in plants. New Phytol 173:677–702

Brown K, Arthur J (2001) Selenium, selenoproteins and human health: a review. Public Health Nutr 4:593–599. https://doi.org/10.1079/PHN2001143

Brown PH, Cakmak I, Zhang Q (1993) Form and function of zinc plants. In: Zinc in soils and plants. Springer, Dordrecht, pp 93–106

Calderini DF, Ortiz-Monasterio I (2003) Are synthetic hexaploids a means of increasing grain element concentrations in wheat? Euphytica 134:169–178

Carvalho SMP, Vasconcelos MW (2013) Producing more with less: strategies and novel technologies for plant-based food biofortification. Food Res Int 54:961–971

Chaudhary J, Khatri P, Singla P, Kumawat S, Kumari A, Vikram A, Jindal SK, Kardile H, Kumar R, Sonah H (2019) Advances in omics approaches for abiotic stress tolerance in tomato. Biology 8(4):90

Chávez AL, Bedoya JM, Sánchez T et al (2000) Iron, carotene, and ascorbic acid in cassava roots and leaves. Food Nutr Bull 21:410–413

Chávez AL, Sánchez T, Ceballos H et al (2007) Retention of carotenoids in cassava roots submitted to different processing methods. J Sci Food Agric 87:388–393. https://doi.org/10.1002/jsfa.2704

Chen L, Chan SY, Cossins EA (1997) Distribution of folate derivatives and enzymes for synthesis of 10-formyltetrahydrofolate in cytosolic and mitochondrial fractions of pea leaves. Plant Physiol 115:299–309

Cömert ED, Mogol BA, Gökmen V (2019) Relationship between color and antioxidant capacity of fruits and vegetables. Curr Res Food Sci 2:1. https://doi.org/10.1016/J.CRFS.2019.11.001

Connolly EL, Campbell NH, Grotz N et al (2003) Overexpression of the FRO2 ferric chelate reductase confers tolerance to growth on low iron and uncovers posttranscriptional control. Plant Physiol 133:1102–1110

Dai JL, Zhu YG, Zhang M, Huang YZ (2004) Selecting iodine-enriched vegetables and the residual effect of iodate application to soil. Biol Trace Elem Res 101:265–276. https://doi.org/10.1385/BTER:101:3:265

Dai JL, Zhu YG, Huang YZ et al (2006) Availability of iodide and iodate to spinach (Spinacia oleracea L.) in relation to total iodine in soil solution. Plant Soil 289:301–308. https://doi.org/10.1007/s11104-006-9139-7

Das A, Laha S, Mandal S et al (2017) Preharvest biofortification of horticultural crops. Elsevier Inc., Amsterdam

de Oliveira VC, Faquin V, Andrade FR et al (2019) Physiological and physicochemical responses of potato to selenium biofortification in tropical soil. Potato Res 62:315–331. https://doi.org/10.1007/s11540-019-9413-8

De Souza JZ, De Mello PR, Silva SLO, Farias TP, Neto JG, Souza Junior JP (2019) Silicon leaf fertilization promotes biofortification and increases dry matter, ascorbate content, and decreases post-harvest leaf water loss of chard and kale. Commun Soil Sci Plant Anal 50(2):164–172

Deshmukh R, Bélanger RR (2016) Molecular evolution of aquaporins and silicon influx in plants. Funct Ecol 30(8):1277–1285

Deshmukh RK, Vivancos J, Ramakrishnan G, Guérin V, Carpentier G, Sonah H, Labbé C, Isenring P, Belzile FJ, Bélanger RR (2015) A precise spacing between the NPA domains of aquaporins is essential for silicon permeability in plants. Plant J 83(3):489–500

Deshmukh RK, Ma JF, Bélanger RR (2017) Role of silicon in plants. Front Plant Sci 8:1858

D'Imperio M, Renna M, Cardinali A, Buttaro D, Santamaria P, Serio F (2016) Silicon biofortification of leafy vegetables and its bioaccessibility in the edible parts. J Sci Food Agric 96(3):751–756

Diretto G, Al-Babili S, Tavazza R et al (2007) Metabolic engineering of potato carotenoid content through tuber-specific overexpression of a bacterial mini-pathway. PLoS One 2:e350

Failla ML, Thakkar SK, Kim JY (2009) In vitro bioaccessibility of $β$-carotene in orange fleshed sweet potato (Ipomoea batatas, lam.). J Agric Food Chem 57:10922–10927

Failla ML, Chitchumroonchokchai C, Siritunga D et al (2012) Retention during processing and bioaccessibility of $β$-carotene in high $β$-carotene transgenic cassava root. J Agric Food Chem 60:3861–3866

Fleshman MK, Lester GE, Riedl KM et al (2011) Carotene and novel apocarotenoid concentrations in orange-fleshed Cucumis melo melons: determinations of $β$-carotene bioaccessibility and bioavailability. J Agric Food Chem 59:4448–4454

Fordyce FM (2013) Selenium deficiency and toxicity in the environment. In: Essentials of medical geology. Springer, Dordrecht, pp 375–416

Fuge R, Johnson CC (1986) The geochemistry of iodine—a review. Environ Geochem Health 8:31–54

Gerber H, Peter H-J, Bürgi E et al (1999) Colloidal aggregates of insoluble inclusions in human goiters. Biochimie 81:441–445

Giuliano G (2017) Provitamin A biofortification of crop plants: a gold rush with many miners. Curr Opin Biotechnol 44:169–180. https://doi.org/10.1016/J.COPBIO.2017.02.001

Giuliano G, Aquilani R, Dharmapuri S (2000) Metabolic engineering of plant carotenoids. Trends Plant Sci 5:406–409. https://doi.org/10.1016/s1360-1385(00)01749-0

Gómez MI, Barrett CB, Raney T et al (2013) Post-green revolution food systems and the triple burden of malnutrition. Food Policy 42:129–138. https://doi.org/10.1016/j.foodpol.2013.06.009

Gómez-Galera S, Rojas E, Sudhakar D et al (2010) Critical evaluation of strategies for mineral fortification of staple food crops. Transgenic Res 19:165–180. https://doi.org/10.1007/s11248-009-9311-y

Goto F, Yoshihara T (2001) Improvement of micronutrient contents by genetic engineering—development of high iron content crops. Plant Biotechnol 18:7–15

Graham RD, Welch RM, Bouis HE (2001) Addressing micronutrient malnutrition through enhancing the nutritional quality of staple foods: principles, perspectives and knowledge gaps. Adv Agron 70:77–142

Grivetti LE, Ogle BM (2000) Value of traditional foods in meeting macro- and micronutrient needs: the wild plant connection. Nutr Res Rev 13:31–46. https://doi.org/10.1079/095442200108728990

Grotz N, Fox T, Connolly E et al (1998) Identification of a family of zinc transporter genes from Arabidopsis that respond to zinc deficiency. Proc Natl Acad Sci 95:7220–7224

Hagenimana V, Carey EE, Gichuki ST et al (1999) Carotenoid contents in fresh, dried and processed sweetpotato products. Ecol Food Nutr 37:455–473. https://doi.org/10.1080/03670244.1998.9991560

Halka M, Smoleń S, Czernicka M et al (2019) Iodine biofortification through expression of HMT, SAMT and S3H genes in Solanum lycopersicum L. Plant Physiol Biochem 144:35–48. https://doi.org/10.1016/J.PLAPHY.2019.09.028

Hanson AD, Gregory JF (2011) Folate biosynthesis, turnover, and transport in plants. Annu Rev Plant Biol 62:105–125. https://doi.org/10.1146/annurev-arplant-042110-103819

Hawkesford MJ, Zhao F-J (2007) Strategies for increasing the selenium content of wheat. J Cereal Sci 46:282–292

Haynes KG, Yencho GC, Clough ME et al (2012) Genetic variation for potato tuber micronutrient content and implications for biofortification of potatoes to reduce micronutrient malnutrition. Am J Potato Res 89:192–198

Hubert B, Rosegrant M, van Boekel MAJS et al (2010) The future of food: scenarios for 2050. Crop Sci 50:33–50. https://doi.org/10.2135/cropsci2009.09.0530

Jomova K, Valko M (2011) Importance of iron chelation in free radical-induced oxidative stress and human disease. Curr Pharm Des 17:3460–3473. https://doi.org/10.2174/138161211798072463

Kanyshkova TG, Buneva VN, Nevinsky GA (2001) Lactoferrin and its biological functions. Biochemist 66:1–7

Kennedy G, Nantel G, Shetty P (2003) The scourge of “hidden hunger”: global dimensions of micronutrient deficiencies. Food Nutr Agric 32:8–16

Korshunova YO, Eide D, Clark WG et al (1999) The IRT1 protein from Arabidopsis thaliana is a metal transporter with a broad substrate range. Plant Mol Biol 40:37–44

Kosambo L (2004) Effect of storage and processing on all trans-$β$ carotene content in fresh Sweet potato (Ipomoea batatas Lam) roots and its products. CIP Funded Res Proj Annu Rep (July 2003–June 2004) Kenya Ind Res Dev Institute, Nairobi, 11p

Kumar A, Lal MK, Kar SS et al (2017) Bioavailability of iron and zinc as affected by phytic acid content in rice grain. J Food Biochem 41:e12413. https://doi.org/10.1111/jfbc.12413

Kumar S, Palve A, Joshi C et al (2019) Crop biofortification for iron (Fe), zinc (Zn) and vitamin A with transgenic approaches. Heliyon 5:e01914

Landini M, Gonzali S, Perata P (2011) Iodine biofortification in tomato. J Plant Nutr Soil Sci 174:480–486

Levenson CW, Morris D (2011) Zinc and neurogenesis: making new neurons from development to adulthood. Adv Nutr 2:96–100

Li H-F, McGrath SP, Zhao F-J (2008) Selenium uptake, translocation and speciation in wheat supplied with selenate or selenite. New Phytol 178:92–102

Lipka AE, Gore MA, Magallanes-Lundback M et al (2013) Genome-wide association study and pathway-level analysis of tocochromanol levels in maize grain. G3: Genes Genomes Genetics 3:1287–1299

Lipkie TE, De Moura FF, Zhao Z-Y et al (2013) Bioaccessibility of carotenoids from transgenic provitamin A biofortified sorghum. J Agric Food Chem 61:5764–5771

Lyons GH, Stangoulis JCR, Graham RD (2004) Exploiting micronutrient interaction to optimize biofortification programs: the case for inclusion of selenium and iodine in the *HarvestPlus* program. Nutr Rev 62:247–252. https://doi.org/10.1111/j.1753-4887.2004.tb00047.x

Lyons G, Ortiz-Monasterio I, Stangoulis J, Graham R (2005) Selenium concentration in wheat grain: is there sufficient genotypic variation to use in breeding? Plant Soil 269:369–380

Mata NL, Radu RA, Clemmons RS, Travis GH (2002) Isomerization and oxidation of vitamin A in cone-dominant retinas: a novel pathway for visual-pigment regeneration in daylight. Neuron 36:69–80. https://doi.org/10.1016/S0896-6273(02)00912-1

Montesano FF, D’Imperio M, Parente A, Cardinali A, Renna M, Serio F (2016) Green bean biofortification for Si through soilless cultivation: plant response and Si bioaccessibility in pods. Sci Rep 6(1):1–9

Mushtaq M, Mukhtar S, Sakina A, Dar AA, Bhat R, Deshmukh R, Molla K, Kundoo AA, Dar MS (2020) Tweaking genome-editing approaches for virus interference in crop plants. Plant Physiol Biochem 147:8

Nandi S, Suzuki YA, Huang J et al (2002) Expression of human lactoferrin in transgenic rice grains for the application in infant formula. Plant Sci 163:713–722

Naqvi S, Zhu C, Farre G et al (2009) Transgenic multivitamin corn through biofortification of endosperm with three vitamins representing three distinct metabolic pathways. Proc Natl Acad Sci 106:7762–7767

Nestel P, Bouis HE, Meenakshi JV, Pfeiffer W (2006) Biofortification of staple food crops. J Nutr 136:1064–1067. https://doi.org/10.1093/jn/136.4.1064

O’Hare TJ (2015) Biofortification of vegetables for the developed world. Acta Hortic 1106:1–8. https://doi.org/10.17660/ActaHortic.2015.1106.1

Ortiz-Monasterio JI, Palacios-Rojas N, Meng E et al (2007) Enhancing the mineral and vitamin content of wheat and maize through plant breeding. J Cereal Sci 46:293–307. https://doi.org/10.1016/j.jcs.2007.06.005

Pinstrup-Andersen P, Hazell PBR (1985) The impact of the green revolution and prospects for the future. Food Rev Int 1:1–25

Pinto E, Ferreira IM (2015) Cation transporters/channels in plants: tools for nutrient biofortification. J Plant Physiol 179:64–82

Prasad AS (2008) Zinc in human health: effect of zinc on immune cells. Mol Med 14:353–357

Prasad R, Shivay YS, Kumar D (2014) Agronomic biofortification of cereal grains with iron and zinc. Adv Agron 125:55–91. https://doi.org/10.1016/B978-0-12-800137-0.00002-9

Ram H, Kaur A, Gandass N, Singh S, Deshmukh R, Sonah H, Sharma TR (2019) Molecular characterization and expression dynamics of MTP genes under various spatio-temporal stages and metal stress conditions in rice. PLoS One 14(5):e0217360

Rana N, Rahim MS, Kaur G, Bansal R, Kumawat S, Roy J, Deshmukh R, Sonah H, Sharma TR (2019) Applications and challenges for efficient exploration of omics interventions for the enhancement of nutritional quality in rice (Oryza sativa L.). CRC Crit Rev Food Sci Nutr:1–17. https://doi.org/10.1080/10408398.2019.1685454

Ratcliffe S, Jugdaohsingh R, Vivancos J, Marron A, Deshmukh R, Ma JF, Mitani-Ueno N, Robertson J, Wills J, Boekschoten MV (2017) Identification of a mammalian silicon transporter. Am J Phys Cell Phys 312(5):C550–C561

Rawat N, Neelam K, Tiwari VK, Dhaliwal HS (2013) Biofortification of cereals to overcome hidden hunger. Plant Breed 132:437–445

Rayman MP (2000) The importance of selenium to human health. Lancet 356:233–241. https://doi.org/10.1016/S0140-6736(00)02490-9

Rayman MP (2012) Selenium and human health. Lancet 379:1256–1268. https://doi.org/10.1016/S0140-6736(11)61452-9

Robinson NJ, Procter CM, Connolly EL, Lou GM (1999) A ferric-chelate reductase for iron uptake from soils. Nature 397:694

Römer S, Lübeck J, Kauder F et al (2002) Genetic engineering of a zeaxanthin-rich potato by antisense inactivation and co-suppression of carotenoid epoxidation. Metab Eng 4:263–272

Saltzman A, Birol E, Bouis HE et al (2013) Biofortification: progress toward a more nourishing future. Glob Food Sec 2:9–17

Sandmann G (2001) Carotenoid biosynthesis and biotechnological application. Arch Biochem Biophys 385:4–12

Santi S, Schmidt W (2008) Laser microdissection-assisted analysis of the functional fate of iron deficiency-induced root hairs in cucumber. J Exp Bot 59:697–704

Scholey D, Belton D, Burton E, Perry C (2018) Bioavailability of a novel form of silicon supplement. Sci Rep 8(1):1–8

Semba RD (1994) Vitamin A, immunity, and infection. Clin Infect Dis 19:489–499

Shimelis H, Laing M et al (2012) Timelines in conventional crop improvement: pre-breeding and breeding procedures. Aust J Crop Sci 6:1542

Sonah H, Deshmukh RK, Labbé C, Bélanger RR (2017) Analysis of aquaporins in Brassicaceae species reveals high-level of conservation and dynamic role against biotic and abiotic stress in canola. Sci Rep 7(1):2771

Stein AJ (2010) Global impacts of human mineral malnutrition. Plant Soil 335:133–154

Strobbe S, Van Der Straeten D (2017) Folate biofortification in food crops. Curr Opin Biotechnol 44:202–211

Terry N, Zayed AM, De Souza MP, Tarun AS (2000) Selenium in higher plants. Annu Rev Plant Biol 51:401–432

Thakkar SK, Huo T, Maziya-Dixon B, Failla ML (2009) Impact of style of processing on retention and bioaccessibility of $β$-carotene in cassava (Manihot esculanta, Crantz). J Agric Food Chem 57:1344–1348

Trumbo P, Yates AA, Schlicker S, Poos M (2001) Dietary reference intakes: vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. J Acad Nutr Diet 101:294

Tuama AA, Mohammed AA (2019) Phytochemical screening and in vitro antibacterial and anticancer activities of the aqueous extract of Cucumis sativus. Saudi J Biol Sci 26:600–604

Tumuhimbise GA, Namutebi A, Turyashemererwa F, Muyonga J (2013) Provitamin A crops: acceptability, bioavailability, efficacy and effectiveness. Food Nutr Sci 4:430

Ueno D, Rombolà AD, Iwashita T et al (2007) Identification of two novel phytosiderophores secreted by perennial grasses. New Phytol 174:304–310. https://doi.org/10.1111/j.1469-8137.2007.02056.x

Van Hal M (2000) Quality of sweetpotato flour during processing and storage. Food Rev Int 16:1–37

Vats S, Kumawat S, Kumar V, Patil GB, Joshi T, Sonah H, Sharma TR, Deshmukh R (2019) Genome editing in plants: exploration of technological advancements and challenges. Cell 8 (11):1386

Velasco I, Bath SC, Rayman MP (2018) Iodine as essential nutrient during the first 1000 days of life. Nutrients 10:290

Vishwakarma K, Mishra M, Patil G, Mulkey S, Ramawat N, Pratap Singh V, Deshmukh R, Kumar Tripathi D, Nguyen HT, Sharma S (2019) Avenues of the membrane transport system in adaptation of plants to abiotic stresses. Crit Rev Biotechnol 39(7):861–883

Wargovich MJ (2000) Anticancer properties of fruits and vegetables. HortScience 35:573–575

Waters BM, Sankaran RP (2011) Moving micronutrients from the soil to the seeds: genes and physiological processes from a biofortification perspective. Plant Sci 180:562–574. https://doi.org/10.1016/J.PLANTSCI.2010.12.003

Welch RM (1995) Micronutrient nutrition of plants. CRC Crit Rev Plant Sci 14:49–82. https://doi.org/10.1080/07352689509701922

Welch RM (2002) Breeding strategies for biofortified staple plant foods to reduce micronutrient malnutrition globally. J Nutr 132:495S–499S. https://doi.org/10.1093/jn/132.3.495s

Welch RM (2005) Biotechnology, biofortification, and global health. Food Nutr Bull 26:S304–S306

Welch RM, Graham RD (2004) Breeding for micronutrients in staple food crops from a human nutrition perspective. J Exp Bot 55:353–364

Weng H-X, Hong C-L, Yan A-L et al (2008a) Mechanism of iodine uptake by cabbage: effects of iodine species and where it is stored. Biol Trace Elem Res 125:59–71. https://doi.org/10.1007/s12011-008-8155-2

Weng HX, Weng JK, Yan AL et al (2008b) Increment of iodine content in vegetable plants by applying iodized fertilizer and the residual characteristics of iodine in soil. Biol Trace Elem Res 123:218–228. https://doi.org/10.1007/s12011-008-8094-y

Weng HX, Hong CL, Xia TH et al (2013) Iodine biofortification of vegetable plants—an innovative method for iodine supplementation. Chin Sci Bull 58:2066–2072. https://doi.org/10.1007/s11434-013-5709-2

White PJ, Broadley MR (2005) Biofortifying crops with essential mineral elements. Trends Plant Sci 10:586–593

White PJ, Broadley MR (2009) Biofortification of crops with seven mineral elements often lacking in human diets - iron, zinc, copper, calcium, magnesium, selenium and iodine. New Phytol 182:49–84

White PJ, Broadley MR (2011) Physiological limits to zinc biofortification of edible crops. Front Plant Sci 2:80

White PJ, Bowen HC, Parmaguru P et al (2004) Interactions between selenium and sulphur nutrition in Arabidopsis thaliana. J Exp Bot 55:1927–1937

WHO (2016) Micronutrient deficiencies. WHO, p 3–5

WHO IGN (2013) Salt reduction and iodine fortification strategies in public health. Report of a joint technical meeting convened by World Health Organization (WHO) and The George Institute for Global Health in collaboration with the International Council for the Control. World Health Organization

Winger RJ, König J, House DA (2008) Technological issues associated with iodine fortification of foods. Trends Food Sci Technol 19:94–101

Zhao FJ, McGrath SP (2009) Biofortification and phytoremediation. Curr Opin Plant Biol 12:373–380

Zhao LI, Yuan L, Wang Z et al (2012) Phytoremediation of zinc-contaminated soil and zinc-biofortification for human nutrition. In: Phytoremediation and biofortification. Springer, Berlin, pp 33–57

Zhu Y-G, Huang Y-Z, Hu Y, Liu Y-X (2003) Iodine uptake by spinach (Spinacia oleracea L.) plants grown in solution culture: effects of iodine species and solution concentrations. Environ Int 29:33–37

Zhu C, Sanahuja G, Yuan D et al (2013) Biofortification of plants with altered antioxidant content and composition: genetic engineering strategies. Plant Biotechnol J 11:129–141