



An Overview on Management of Micronutrients Deficiency in Plants Through Biofortification: A Solution of Hidden Hunger

Pradeep Kumar Yadav, Anita Singh, and S. B. Agrawal

Abstract

Nowadays, malnutrition is one of the major problems, especially for the poor population of developing countries. The major staple crops are found to be deficient in some mineral elements, especially the micronutrients that result in the problem of hidden hunger. There are several promising strategies that are applied in agricultural fields to solve this problem. They enhance the bio-available concentrations of micronutrients in edible crops. One of the recent strategy is biofortification, which can be used to increase the content and/or **bioavailability** of vital nutrients in food crops through genetic (genetic transformation/plant breeding) and agronomic pathways (application of nutrient fertilizers). These strategies provide more nutritious diets to more people. Along with the traditional agricultural practices, the “omics” technologies can modify the crops by genetic transformation that improves the uptake, transport, and mineral accumulation in hybrid plants. This chapter has detail information about the nutrient constituents and its uptake in the plants along with a critical comparison of the several strategies that have been developed to enhance mineral levels and bioavailability of micronutrients in most of the important food crops.

P. K. Yadav

Center of Advanced Study in Botany, Institute of Science, Banaras Hindu University, Varanasi, Uttar Pradesh, India

A. Singh (✉)

Center of Advanced Study in Botany, Institute of Science, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Department of Botany, Institute of Science, Banaras Hindu University, Varanasi, Uttar Pradesh, India

S. B. Agrawal

Laboratory of Air Pollution and Global Climate Change, Center of Advanced Study in Botany, Institute of Science, Banaras Hindu University, Varanasi, Uttar Pradesh, India

The use of biofortified crops should be promoted by educating the farmers by government agencies, so that they can be included in their diet to solve the problem of malnutrition up to certain extent.

Keywords

Food crops · Nutrients · Malnutrition · Agronomic · Biofortification

8.1 Introduction

Plants require several mineral elements or nutrients available in nature for their proper growth and development (Watanabe et al. 2007). These mineral elements are classified in to macro and micronutrients. Macronutrients are used in large concentration and further divide into structural (carbon: C, hydrogen: H, nitrogen: N), primary (nitrogen: N, phosphorus: P, potassium: K), and secondary nutrients (calcium: Ca, magnesium: Mg, sulfur: S). The micronutrients like boron, chlorine, manganese, iron, zinc, copper, and molybdenum are required in very less amount. These elements are naturally present in the soil, and taken up by the roots in ionic forms only. However, with the frequent uses of fertilizers, over-cropping, and application of waste water and sewage sludge, the availability of these elements get disturbed. The practices of waste water irrigation and sewage sludge application also lead to the accumulation of several toxic metals. They ultimately affect soil characteristics and availability of different nutrients due to the competition among heavy metals and mineral elements. The deficiencies of micronutrients not only affect the production of crops but also contribute malnutrition due to poor nutritional quality of food crops. It often results into invisible health problems, hence termed as hidden hunger (de Valenca et al. 2017). Mineral nutrients are mainly absorbed by the roots from soil system, however many factors have their effect on nutrient attainment. Sometimes, mineral elements are not in their available forms and also soil properties such as pH, conductivity, bulk density, etc. have their effect on nutrient uptake (Morgan and Connolly 2013).

To certain extent, to manage the deficiency of the micronutrients and to maintain the balance among essential elements, the plants try to cope up by themselves. However, at the time of the severe deficiency of micronutrients, the plants cannot cope with the condition by themselves only; in that case several other strategies are applied to deal with the situation. In this direction, biofortification is one of the processes that can be applied in different ways (agronomic or genetic) to increase the [bioavailability](#) and solubility of essential nutrients (Bouis and Saltzman 2011). Genetic biofortification can be achieved by [genetic engineering](#) or classical plant breeding (Saltzman et al. 2013), whereas agronomic biofortification can be achieved through [fertilizer](#) application either as a soil solution/foliar spray or through fertigation. With this process the essential minerals can be added in the common diets of population by increasing the solubility and availability of nutrients. From an economic point of view, biofortification is also one time investment because it offers

a cost-effective, long term, and sustainable approach to manage the hidden hunger. The basic theme of biofortification should be that all the malnourished and poor families get all the essential micronutrients through their diet. There are some organizations like World Health Organization and the counseling Group on International Agricultural Research (CGIAR) have developed several nutritionally enhanced high-yielding biofortified crops (Jha and Warkentin 2020).

With the above context, the present chapter has detailed information on micronutrient uptake and includes different ways to manage deficient level of micronutrients in food crops. It also consists of discussion on biofortification processes in detail to show their positive impact on elemental composition of staple crops.

8.2 Uptake and Distribution of Micronutrients in the Plants

Plants naturally absorb different mineral elements from the soil through their root system. They can only take the element in their available forms, i.e., in ionic forms (Table 8.1). The availability of nutrients is dependent upon several soil characteristics like moisture content, bulk density, texture, organic matter content, pH, cation exchange capacity (CEC), and soil biological properties. The nutrient uptake is the natural process through which they enter in to the plants either by roots or by the leaves. For the uptake and distribution of nutrients, several physiological and molecular processes are involved. Among all the micronutrients, Cl and Mo, absorbed as anions, “B” is in neutral or anionic form, Mn, Cu, Zn, and Ni are in divalent cations and Fe can be absorbed as both divalent and trivalent cations (Lambert et al. 2008). As the cytoplasm of plant cell is negatively charged so the type and number of charges play important role in the transportation of micronutrients. The two common pathways include passive and active absorption. In passive absorption, minerals are absorbed without the direct expenditure of metabolic energy. If the transported elements carry a net charge, its movement is influenced by both its concentration gradient and membrane potential. Passive transport can be occurred either through simple diffusion and facilitated diffusion.

8.2.1 Simple Diffusion

During simple diffusion, a mineral element simply dissolves in phospholipid bilayer, diffuses across it and no membrane proteins are involved and the direction of movement determined simply by the relative concentration of molecule inside and outside of the cell.

8.2.2 Facilitated Diffusion

It involves the movement of mineral solute along the concentration gradient. Facilitated diffusion either occurs through carrier protein or channel protein. It

Table 8.1 The available forms and function of micronutrients

Micronutrients	Available form	Transporters involved for uptake	Function
Boron	Absorbed as BO_3^{3-} or $\text{B}_4\text{O}_7^{2-}$	NIPs and BOR1	Boron is required for uptake and utilization of Ca^{2+} membrane functioning, pollen germination, cell elongation, cell differentiation, and carbohydrate translocation.
Molybdenum	Obtain in the form of molybdate ion (MoO_4^{2-})	MOT1, MOT2	It is a component of several enzymes, including nitrogenase and nitrate reductase both of which participate in nitrogen metabolism.
Chlorine	Absorbed in the form of chloride anion (Cl^-)	CLC, CCC	Along with Na^+ and K^+ it helps in determining the solute concentration and the anion-cation balance in cells. It is essential for the water-splitting reaction in photosynthesis, a reaction that leads to oxygen evolution.
Manganese	Absorbed in the form of manganous ion (Mn^{2+})	NRAMP, ZRT/IRT, YSL	It activates many enzymes involved in photosynthesis, respiration, and nitrogen metabolism. The best defined function of manganese is in the splitting of water to liberate oxygen during photosynthesis.
Zinc	Obtain zinc as Zn^{+2}	ZIPs, HMAs, YSLs MTPs, FRD3, ZIF1, NASs	It activates various enzymes, especially carboxylases. It is also needed in the synthesis of auxin.
Copper	Absorbed as cupric ion (Cu^{2+})	COPT1, COPT2, COPT3 and COPT4, ZIP2 and ZIP4	It is essential for the overall metabolism in plants. Like iron, it is associated with certain enzymes involved in redox reactions.
Iron	Plant obtains iron in the form of ferric ion (Fe^{2+} , Fe^{3+})	ZIPs, NRAMPs, YS1 and YSLs	It is an important constituent of proteins involved in the transfer of electrons like ferredoxin and cytochromes. It is reversibly oxidized from Fe^{2+} to Fe^{3+} during electron transfer. It activates catalase enzyme, and is essential for the formation of chlorophyll.

allows polar and charged mineral elements such as carbohydrate, amino acid, nucleoside, and ion to cross the plasma membrane.

Active absorption of minerals directly utilized metabolic energy and during this process, minerals are absorbed in ionic forms against the concentration gradient. The energy required for this process is obtained from the cell's metabolism either directly or indirectly.

The micronutrients are present in very low concentration and available in the charged form so they cannot move across cell membranes with passive absorption only. They can also enter into the roots actively with the help of ATP as energy source in presence of specific proteins (transport ATPase, ABC transporter, etc.). They are present in the plasma membranes of endodermal cells that control the entry of mineral ions into the xylem cell based upon their type and quantity. The transporter proteins involved in the uptake and distribution pattern of some specific micronutrients are discussed below:

1. Boron (B)

Plant absorbed boron (B) as BO_3^{3-} or $\text{B}_4\text{O}_7^{2-}$. When boron is present in sufficient amount then it is absorbed by simple passive diffusion but under B deficient condition, it is absorbed by the plant with the help of transporters. The molecular genetic studies revealed that there are two types of B transporters, (nodulin-26-like intrinsic proteins) NIPs and BOR1 in *Arabidopsis thaliana* (Takano et al. 2006). NIP5;1 and a boric acid channel facilitate influx of B in the root cells. It is observed that NIPs help in the transfer of B from xylem–phloem to young growing tissues (Tanaka et al. 2008). In *Arabidopsis thaliana* L. the first B transporter, AtBOR1 was reported and studied most intensively. In rice (*Oryza sativa* L), OsBOR1 also helps in the uptake of B just like AtBOR1 (Nakagawa et al. 2007). Kato et al. (2009) reported that under B deficient condition the NIP5;1 transporter gets over expressed and improved elongation of root. With the over-expression of both the transporters such as BOR1 and NIPs, the *Arabidopsis thaliana* plants can be able to grow in B deficient soil.

2. Molybdenum (Mo)

In plants, molybdenum is absorbed as molybdate (MoO_4^{2-}). Due to high degree of similarity with SO_4^{2-} , the uptake and distribution of molybdate (MoO_4^{2-}) are supported by the transporters involved in sulfate transporters (Dudev and Lim 2004). The first molybdate-specific transporters (MOT1) were identified in *Arabidopsis thaliana* (Tomatsu et al. 2007). MOT1 is a relative of the sulfate transporter superfamily (Buchner et al. 2004), but does not appear to transport sulfate. The role of MOT1 in molybdate uptake is still unclear as results suggest MOT1 is localized to mitochondria (Baxter et al. 2008). Along with this MOT1, another molybdate transporter MOT2 has also been identified from *Arabidopsis* that also belongs to the sulfate family (Gasber et al. 2011). It localizes in the vacuolar membrane and helps in exporting stored molybdate from the vacuole into the cytosol and finally into maturing seeds. Another molybdate transporter also denoted as MOT2 has been reported from *Chlamydomonas* that does not belong to the sulfate transporter family (Tejada-Jiménez et al. 2011). Researches to find out the homolog of this transporter in higher plants are still under process to understand the uptake of molybdate at the root: soil interface in detail.

3. Chlorine (Cl)

The chlorine is absorbed as chloride (Cl^-) and it is transported via H^+ /anion symporters. They help in the Cl^- uptake and release it into the xylem cell (Roberts 2006). Putative H^+ /halide transporters include ATP binding cassette (ABC) protein super family and chloride channel (CLC) transporter family (Marmagne et al. 2007; Verrier et al. 2008). At the same time, Na:K/Cl symporters also help in the uptake of Cl^- by the cation chloride co-transporter (CCC) gene family (Colmenero-Flores et al. 2007). Some organic acid transporters also help in the halide fluxes in the plants (White 2001). The Cl^- is mainly accumulated in roots and leaves and little is redistributed via the phloem to fruits or seeds (Muramatsu et al. 1995).

4. Manganese (Mn)

Manganese (Mn) is only available in its reduced form (Mn^{2+}) and can be able to transport from soil to root and then to the shoot. In alkaline soil, availability of Mn is decreased by converting Mn^{2+} into insoluble Mn oxides (MnO_x) (Stumm and Morgan 1996). There are several transporters involved in the homeostatic network of Mn in plants. The Natural Resistance Associated Macrophage Protein (NRAMP) family, the Zinc-Regulated Transporter/Iron-Regulated Transporter (ZRT/IRT)-related Protein (ZIP) family, and the Yellow Stripe-Like (YSL) are involved in the transportation of Mn^{2+} into the cytosol (Alejandro et al. 2020).

5. Zinc (Zn)

In soil solution, Zn is present in very low amount but has critical importance for plants. The substantial amount of Zn reaches to the xylem cells of root apoplastically (Broadley et al. 2007). It can be transported to the plasma membrane of root cell in the form of Zn^{2+} or as a complex of Zn with phytosiderophore (Ismail et al. 2007). The influx of most of the Zn^{2+} into the cytoplasm mediated by ZIPs (ZIP1, ZIP3, and ZIP4; Palmgren et al. 2008), and the Yellow Stripe-Like (YSL) family proteins help in uptake of Zn by the formation of Zn-phytosiderophore complexes (Suzuki et al. 2008). In the xylem cell, the transportation of Zn occurs in the form of Zn^{2+} , by binding with organic acids like histidine or nicotianamine (Broadley et al. 2007; Palmgren et al. 2008). In the leaf and phloem cell, influx of Zn^{2+} is mediated by the members of the ZIP family (Ishimaru et al. 2005). In addition, YSL proteins may load Zn into the phloem, where Zn is transported as a Zn-NA complex, or as a complex with small proteins, to sink tissues (Waters and Grusak 2008). Although Zn mobility in the phloem is generally considered to be low, this may not always be the case (Welch 2002; Haslett 2001). During Zn deficiency, uptake, sequestration, and redistribution of Zn get increased by the over-expression of genes responsible for Zn uptake in the plant. These genes encode different proteins such as ZIPs, HMAs, YSLs MTPs,

FRD3, ZIF1, NASs and it also increases the biosynthesis of phytosiderophores to enhance the Zn uptake (Milner and Kochian 2008).

6. Copper (Cu)

Copper can be absorbed as Cu^+ and Cu^{2+} with the help of copper transporters (CTR) such as COPT1, COPT2, COPT3, and COPT4 and by ZIPs (ZIP2 and ZIP4), respectively (Grotz and Guerinot 2006). Expressions of these transporters get up-regulated under Cu deficient condition (Wintz et al. 2003). Cu is loaded into the xylem cell and transported in a Cu^{2+}NA complexed form (von Wiren et al. 1999). In phloem it is by YSL protein and transported as Cu-NA complex (DiDonato Jr et al. 2004). This protein helps in the transportation of Cu-NA complexes and Cu^{2+} and Fe^{2+} cations in their free form (Wintz et al. 2003).

7. Iron (Fe)

The uptake of iron (Fe) is dependent upon types of plants species. In non-graminaceous species, the plant's roots release some organic and phenolic compounds to acidify the rhizospheric zone that increase the Fe^{+3} concentrations in soil solution. Then, with the help of ferric reductases (encoded by members of the ferric reductase oxidase; FRO, gene family), Fe^{+3} get reduced to Fe^{+2} in the epidermal cell of root (Mukherjee et al. 2006). Next, the members of different transporter proteins help in the influx of Fe^{2+} to root cells such as zinc-regulated transporter (ZRT)-, iron-regulated transporter (IRT)- protein (ZIP) family (AtIRT1 in *Arabidopsis*) (Ishimaru et al. 2005). In contrast to this, graminaceous spp. release structural derivatives of mugineic acid, i.e., phytosiderophores that bind with Fe^{3+} and whole complex is absorbed by root cells (Ishimaru et al. 2005). Again, within the xylem, Fe is transported as a Fe^{3+} citrate complex (Abadía et al. 1984; Mukherjee et al. 2006). In *Arabidopsis*, it was reported that one of the member of the multidrug and toxin efflux (MATE) family; FRD3 is present in the root pericycle and help in the transportation of Fe from root to shoot in the form of citrate complex (Puig et al. 2007) and members of the ZIP family help in the uptake of Fe^{2+} by shoot cells. During Fe deficiency, expression of genes ferric reductase oxidase (FROs) get upregulated that encode proteins responsible for the uptake and redistribution of Fe. These include genes encoding ZIPs, NRAMPs, YS1, and YSLs (Grotz and Guerinot 2006; Kramer et al. 1996; Stacey et al. 2008) and enzymes such as nicotianamine synthase (NAS), and phytosiderophores to synthesize nicotianamide (NA) and help in more uptake of iron by the plant's root cell

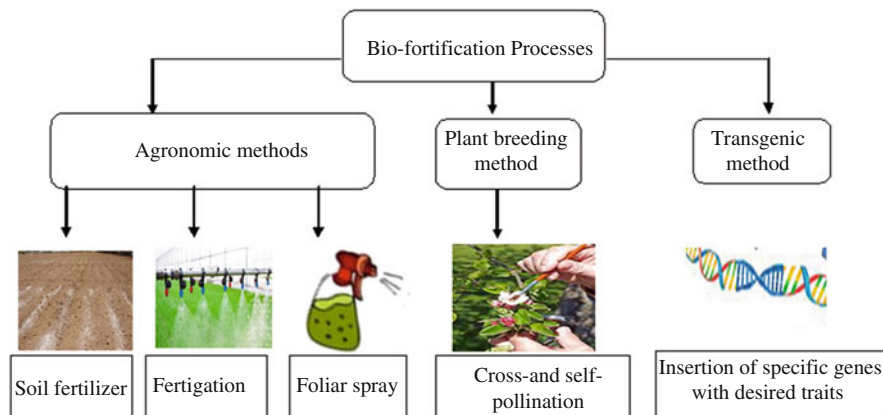


Fig. 8.1 Different ways of biofortification

8.3 Different Ways of Biofortification to Manage Micronutrient Deficiency in Plants

For biofortification, there are three main methods used to manage nutrient deficiency in the plants such as agronomic method, plant breeding method, and transgenic method (Fig. 8.1). Each one is discussed further in detail.

8.3.1 Agronomic Approach

Agronomic approach includes application of fertilizers that helps in increasing nutritive values of plants without modifying their genetic setup (Almendros et al. 2015). This technique is able to provide efficient micronutrients concentrations in edible crops and it is one of the immediate and effective approaches (de Valenca et al. 2017). Soil deficiency is reflected by the poor nutrients composition of crops. This problem is aggravated by growing cereal crops on soils potentially deficient in nutrients. Nutrient deficiency in humans is also seen mainly in those regions where crops are grown in nutrient deficient soil (Bilski et al. 2012). Intervention of new agricultural approaches to improve production of micronutrient-rich foods is one of the main areas of research and competent strategy to supplement the nutrients in food products (Pandey et al. 2016). The agronomic biofortification of cereal crops appears to be a rapid and simple solution to manage the deficiency of important elements in soils and plants. With this approach, one should take care that the over fertilization can be toxic to the plants. The potential of agronomic fortification is strongly related with micronutrient bioavailability at three stages: from soil to plants, from plants root to the edible parts, and from edible parts to humans. Agronomic biofortification has positive impact on plant characteristics and nutritional status of plants. In

combination with NPK, organic fertilizers, the micronutrients fertilization can improve crop varieties and that particularly highlights the importance of integrated soil fertility management.

Biofortification through agricultural methods includes application of nutrients directly in the soil and water that affect the health status of crops and provide quality food to the human being of plants. Agronomic biofortification is easy and cost-effective technique but more awareness and detail study is required to understand about the forms of fertilizer, mode of applications, and impact on other environmental components.

8.3.1.1 Application of Fertilizer in Soil and Irrigation Water

The simplest way to increase the density of nutrients in the edible crops is by enhancing their availability through different forms of fertilizers, so that plants can uptake the nutrient from soil in a more efficient way (Almendros et al. 2015). The types of nutrients source and soil characteristics have a great influence on agronomic biofortification and that consequently affect the qualitative and quantitative characteristics of food crops. Soils show variation in their mineral composition and phytoavailability of nutrients basically based upon several factors such as pH, water holding capacity, cation exchange capacity of soil, specific surface area, surface charge density, as well as cation exchange capacity (Pinto and Ferreira 2015). Based on adsorption–desorption characteristics of soils, the application of fertilizers leads to enhancement in the concentration of nutrients in the plant's parts (Dai et al. 2009). The composition of fertilizers play important role in providing nutrients as well interactions among them can have positive neutral or even negative effects on yields and nutrient use efficiencies (Saha et al. 2015; Rietra et al. 2015).

Inorganic and Organic Fertilizers

With rising expectations toward agricultural production, the importance of micronutrient fertilization has increased tremendously. Soil nutrients especially the microelements are insufficient to meet increased crop requirements that affect both yields and quality of the crops. The standard NPK-based fertilization must often be supplemented by the deficient micronutrients. There are several inorganic forms of micronutrients that are applied with NPK fertilizers to support the growth of plants (Table 8.2a). Within the agronomic biofortification practice, the most common method to enhance the micronutrient levels in the field soil is by adding fertilizers in the form of inorganic salts. It brings good results based upon kind of supplemented micronutrient and the chemical properties of fertilized soil. Zinc sulfate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) and copper sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) are the most tested fertilizers, it has been observed that inorganic fertilizers applied after seed sowing lead to better yield as compared to pre-sowing soil fertilization (Smoleń and Sady 2012). Another interesting approach for the biofortification is the application of inorganic salt to obtain a new formulation, as in the case of Se-enriched peat. Although Se is not required by the plants but its certain level is important for the metabolic activities of human and animals. The peat was enriched by thoroughly

Table 8.2a Inorganic form of nutrients applied in the soil along with the NPK fertilizers

Nutrient	Salt	Form
Zinc (Zn)	Zinc sulfate	ZnSO ₄ ·7H ₂ O
	Chelate	Zn EDTA
	Zinc oxide	ZnO
Copper(Cu)	Copper sulfate	CuSO ₄
Iron (Fe)	Ferrous sulfate	FeSO ₄ ·7H ₂ O
Manganese (Mn)	Manganese sulfate	MnSO ₄ ·H ₂ O
Chlorine (Cl)	Potassium chloride	KCl

Source: Modified from Jones and Jacobsen (2009)

mixing it with a solution of sodium selenite and then applied during the pre-transplanting stage (Businelli et al. 2015).

Some plants and peat, in the presence of high level of inorganic Se, can metabolize and accumulate it in the form of organic derivatives. This process is important for the plant because it reduces the toxicity of the chalcogen and, at the same time, when the bioaccumulation occurs in edible tissues, it allows enrichment of food with Se that is good for the humans and animals. Moreover, Se biofortification also increases secondary metabolites production in human beings when consumed with the diet. Therefore, biofortification strategies applied to produce Se-enriched foods could help to overcome Se deficiency and its implications on human health and it also improve the nutraceutical (substance, which has physiological benefit or provides protection against chronic disease) value of food.

Similarly, organic fertilizers are also the source of micronutrient for plants. Earlier during the agricultural practices, it was observed that crop yields could be enhanced with the addition of animal manure or plant debris to the soil. A new study reveals that Neolithic farmers when the first developments of farming appeared used livestock manure to enhance crop yields (Bogaard et al. 2013). This practice is in continuation with regular additions of organic matter (from different sources), which is used mainly in organic and integrated farming systems (Kizos et al. 2010). Organic fertilizers are materials whose basic ingredient is organic matter (Adegoke et al. 2016). They traditionally derived from animal excreta (livestock manure, slurry, poultry feces) and vegetable matter (straw, green manures). Naturally occurring organic fertilizers include peat, seaweed, and guano (accumulated excrement of seabirds and bats). Guano is also an effective fertilizer due to its exceptionally high content of nutrients (Hazra 2016). Recently, municipal and industrial wastes are taken into account as organic renewable resources to improve the nutritional status of plants. The most important organic materials that accumulate in industrial countries are sewage sludge, bio-compost and by-products from the food and foodstuff industry provide several nutrients (Table 8.2b). Waste from the food and luxury item industries can also be applied in agricultural fields as organic waste. These organic wastes act as an important secondary source of micronutrients and their availability depend upon soil organisms (Jones and Jacobsen 2009).

Table 8.2b Micronutrient content of selected organic fertilizers in mg/kg

Organic fertilizers	Fe	Cu	Mn	Zn	Source
Sewage sludge	2275– 3322	7– 11	100– 287	68– 177	Tennakoon and Bandara (2003)
Green manure (Acacia)	870–994	7–9	78–92	54– 610	Tennakoon and Bandara (2003)
Cattle manure	1075	880	247	44	Uyanoz (2007)
Pig manure	1416	502	367	563	Li et al. (2009)
Rice straw	225	3.73	467	49.6	Li et al. (2009)
Sheep manure compost	1248.9	4.0	45.6	68.9	Wang et al. (2016)

Biofertilizers

Application of biofertilizers is also one of the ways of agronomic biofortification to raise nutrients content in the plants by increasing the solubilization and mobilization rate of elements (Almendros et al. 2015). Biofertilizers can be described as diverse groups of soil-borne microbes, such as root endophytic fungi, mycorrhizal fungi, plant growth-promoting rhizobacteria, and rhizobia that exert positive effects on plant yields and survival through direct and plant-mediated mechanisms. They help in the nitrogen fixation, solubilize the insoluble minerals, produce phytohormones, and also protect the plants from pathogens (Olivares et al. 2015). Microorganisms can be used as substitutes for various chemical fertilizers and improve plant nutrition and health. Usually biofertilizers do not contain a single culture of beneficial microorganism but a mixture of different microorganisms. Soil particularly the rhizospheric zone contains some bacterial species that promote growth of the plants and collectively termed as Plant Growth-Promoting Rhizobacteria (PGPR; *Rhizobia* spp. And *Frankia* spp.). PGPR can facilitate acquisition of resources and modulate the levels of plant hormones. They are able to provide resistance to the plants against various pathogenic agents. The PGPR consortium, named “BioPower” consist of two *Azospirillum lipoferum* strains, two *Pseudomonas* sp. strains and one *Agrobacterium* sp. strain. It has been found to increase the availability of Zn in rice crop (Tariq et al. 2007). Rana et al. (2012) have applied three rhizobacterial strains: *Bacillus* sp., *Providencia* sp., and *Brevundimonas* sp., applied along with NPK fertilizers. The study showed significant enhancement in Fe, Cu, Zn, and Mn content in wheat plant. The application of *Pantoea dispersa* MPJ9 and *Pseudomonas putida* MPJ6 increased the Fe content in mung beans by 3.4 times under Fe deficient soil by producing Fe chelating agent, i.e., siderophores (Ghosh et al. 2019). Ramesh et al. (2014) have inoculated two strains of *Bacillus aryabhattai* (MDSR7 and MDSR14) in Zn deficient soil that improved the Zn uptake in soybeans and wheat crops. Gopalakrishnan et al. (2016) have studied effect of seven strains of PGPR and reported that among all *Enterobacter ludwigii* and *Acinetobacter tandoii* SRI-229 strains showed significant enhancement in Fe, Zn, Cu, and Mn concentrations in chickpeas and pigeon peas. Recently, Singh and Prasanna (2020) have coated chickpea seeds with Zn solution along with Zn solubilizer PGPR, *Enterobacter* sp. MN17 that improved the bioavailability of Zn and consequently the grain yield.

Different PGPR strains have differential abilities to fix or solubilize nutrients within the rhizosphere for promoting growth and yield of the plants (Amaya-Gómez et al. 2020).

The effectiveness of micronutrient fertilizer application on crop biofortification is influenced not only by the fertilizer type but also by the application method (Mao et al. 2014; Melash et al. 2016; de Valenca et al. 2017). In crop plants, micronutrients may be applied to the soil as foliar spray seed treatments, or through fertigation (Farooq et al. 2012; Singh and Prasad 2014; Smoleń et al. 2016). The decision for the selection of method depends upon the requirement of specific nutrients and form of fertilizers (Pankaj and Dewangan 2016). Some of the micronutrients like Fe and Mn were applied through foliar application and that improved the growth of plants (Narwal et al. 2012). Organic fertilizers are spread uniformly in the field and incorporated several days before planting (Piechota et al. 2014). Micronutrient salts can be applied as a granular material or dissolved in liquid fertilizers (Pagani et al. 2013).

Another way of fertilizer application is with irrigation water, i.e., Fertigation, it is made up of two words, i.e., fertilization and irrigation. In this, fertilizer is applied with the irrigation water through drip irrigation method (Bell and Dell 2008). Through this process fertilizer solution is distributed evenly with irrigation water. With this mode, the availability of nutrients is increased mainly in the rhizospheric zone. During this process, only liquid fertilizer and the fertilizers soluble in water are used. Fertigation is practiced extensively in commercial agriculture and horticulture. It is used to add additional nutrients or to manage nutrient deficiencies detected in plant tissue. It is usually practiced on the high-value crops such as vegetables, fruit trees, and cereals for the purpose of biofortification. The nutrient used in fertigation must be highly soluble in water like monoammonium phosphate (Nitrogen and Phosphorus), poly feed (Nitrogen, Phosphorus, and Potassium), Multi K (Nitrogen and Potassium), Potassium sulfate (Potassium and Sulfur). Some of the nutrients used in fertigation are as follows:

- Ammonium nitrate
- Ammonium sulfate
- Urea
- Monopotassium phosphate
- Potassium sulfate
- Potassium nitrate
- Potassium chloride
- Diammonium phosphate

Through fertigation, the water and fertilizer are evenly supplied to the crops, so there is more possibility of getting 25–50% higher yield. It also minimizes the amount of fertilizers applied and the time, labor, and energy utilized during this process. It leads to the reduction in soil erosion as here nutrients are applied through the drip irrigation (Khalid et al. 2015).

Agronomic Fortification Through Foliar Application

In addition to nutrients being added to the soil as fertilizers, some mineral nutrients can be sprayed to the leaves and this process is known as foliar application (Mortvedt 1985). Over to soil fertilization, the foliar spray is found to be more beneficial by enhancing the nutrient uptake and their allocation in the edible plant parts (Lawson et al. 2015; de Valenca et al. 2017). Other advantages of foliar sprays are: uniform distribution is easily obtained, response to the applied nutrient is almost immediate; therefore, deficiencies can be managed easily. Foliar feeding is associated with higher yields and better quality of fruits. The efficiency of nutrient uptake is increased by 8–9 times when nutrients are applied as a foliar spray, as compared to the soil application (de Valenca et al. 2017).

Foliar fertilization has the ability to improve the efficiency and utilization of nutrients, required by the plant for their maximum growth and yield. The main advantage of foliar fertilization is the immediate uptake of applied nutrients. The most important use of foliar application is that only limited amount of micro and macronutrients are applied, which do not cause any kind of phytotoxicity (Oosterhuis and Weir 2010).

It also makes available those nutrients like Zn and Fe which are not available to the plants through root uptake. A foliar application is recommended when environmental conditions limit the uptake of nutrients by roots such as variation in pH, moisture and nutrient imbalances in soil, etc. The availability of micronutrient is decreased at high soil pH and under such circumstances the more efficient way to supply micronutrients to the plant is foliar spray rather than soil application (Adams 1984).

Zhang et al. (2012) have also reported that foliar application of Zn is more effective than soil application. The study showed that foliar application of 0.4% $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ resulted 58 and 76% increase in Zn concentration, respectively, in grain and flour of wheat. The foliar Zn application provides an effective way to enhance dietary Zn in the edible products derived from wheat. Rugeles-Reyes et al. (2019) have also reported that the application of zinc (1.5 kg ha^{-1}) as foliar spray leads to 279% enhancement in the supply of Zn from plant to the humans as compared to the control. So, the foliar applications of the nutrient are found to be effective in increasing Zn contents in the plant leaf.

8.3.2 Plant Breeding Technology

Biofortified crops can be developed by breeding methods, but it is possible only when sufficient genetic variation is present in crop populations for the desired traits. Conventional plant breeding through cross- and self-pollination strategies plays major role in improving agricultural productivity. During conventional plant breeding, in order to produce desirable agronomic traits, the parent plants having high nutrients are crossed over several generations with recipient one (Garg et al. 2018). The most significant, systematic and symbolic program of biofortification through conventional breeding is “Harvest Plus.” The main goal of *Harvest Plus program* is

to develop and promote the production of biofortified food crops that improve the public health. It basically increased levels of three nutrients (iron, zinc, and pro-vitamin A) in seven staple crops (beans, cassava, maize, rice, wheat, sweet potato, and pearl millet) (Nestel et al. 2006). The Harvest Plus programme is funded principally by grants from foundations, governments, and international agencies, started in 2003, by the Consultative Group on International Agricultural Research (CGIAR). The main vision of this program is to provide more nutritious food to 1 billion people by 2030.

The success rate of plant breeding technique to produce fortified crop is dependent upon their acceptance by the farmers as well as absorption rate of micronutrients in the consumers (Bouis 2003). Only the staple food varieties whose seeds are micronutrient rich are feasible for plant breeding. The micronutrient efficient varieties developed by plant breeding can grow deeper in the mineral deficient soil. The roots of new varieties are more efficient in mobilizing the external minerals and are able to utilize the moisture and nutrients present in the subsoils. This will reduce the application rate of fertilizer as well as irrigation. The efficient uptake of minerals from soil and their loading into the grains lead to higher yield. So, the farmers can easily accept new varieties with mineral dense seeds and higher yield (Bouis 2003). There are several studies where Zn and Fe concentrations are estimated in different varieties of plants to find out the best variety with their highest concentration of nutrients for breeding program (Velu et al. 2015; Garg et al. 2018; Jha and Warkentin 2020). Researchers at IIRRI have studied six sets of genotypes ($n = 939$) and evaluated Fe and Zn concentrations. The Fe concentration ranged from 7.5–24.4 $\mu\text{g g}^{-1}$ for Fe, and Zn concentration ranged from 13.5–58.4 $\mu\text{g g}^{-1}$. Among all varieties, Jalmagna, Zuchem, and Xua Bue Nuo had highest concentration of Fe and Zn. The F2-derived populations of these varieties showed that this trait is not found to be pleiotropic for grain-Fe or-Zn concentrations so can be used for further breeding program. Over the common rice variety, Jalmagna is the traditional variety that had nearly 40% more iron concentration. Under Harvest Plus program, Bangladesh Rice Research Institute has developed world's first rice varieties (BRRIdhan 62, BRRIdhan 72, and BRRIdhan 64) with high Zn concentrations (20–22 ppm). In India and Philippines, by crossing a variety having high yield (IR72) with Zawa Bonday; a tall variety, an improved line (IR68144-3B-2-2-3) was developed in order to enhance the level of Fe (21 ppm) in the grain (Palanog et al. 2019).

Similarly, wide range of wheat germplasm is being studied at International Maize and Wheat Improvement Center (CIMMYT; Spanish acronym) with respect to the Fe and Zn concentrations in the whole grain. Among all wheat germplasm, *Triticum dicoccum* with highest concentrations of Fe and Zn can be used for further study (Welch and Graham 2002). Through collaboration with Banaras Hindu University (BHU), Uttar Pradesh (UP), India, in 2014, six varieties of wheat named as BHU 1, BHU 3, BHU 5, BHU 6, BHU 7, and BHU 18 with high concentration of Zn (4–10 ppm) were released under Harvest Plus program (Velu et al. 2015). One variety (WB2) with high concentration of Zn and Fe has been developed by Indian Institute of Wheat and Barley Research, India (Chatrath et al. 2018). Along with this,

four varieties (NR 419, 42, 421, and Zincol) and one variety (PBW1Zn) of wheat with high Zn were released by University of Agriculture Faisalabad, Pakistan (Ohly et al. 2019) and Punjab Agricultural University, India (Bhati et al. 2016), respectively. The degrees of genetic variability for Zn and Fe contents in bean seeds have been studied by the researchers of the International Center for Tropical Agriculture (CIAT). More than 1000 varieties of common beans are collected and showed varied levels of Fe from 34–89 $\mu\text{g g}^{-1}$ and of Zn from 21–54 $\mu\text{g g}^{-1}$. Due to presence of sufficient genetic variability, the Fe and Zn concentration can be increased significantly by plant breeding technology (Graham et al. 1999). Several varieties of common bean with high Fe content have been developed under HarvestPlus in Rwanda, Eastern Africa Democratic Republic of Congo also released ten biofortified varieties (COD MLB 001, COD MLB 032, HM 21–7, RWR 2245, PVA 1438, COD MLV 059, VCB 81013, Nain de Kyondo, Cuarentino, Namulenga) with high Fe concentration (Andre et al. 2007). With the help of ICARDA, HarvestPlus biofortification program, some varieties of lentil Barimasur-4, Barimasur-5, Barimasur-6, Barimasur-7, and Barimasur-8 in Bangladesh and ILL 7723, Khajurah-1, Khajurah-2, Shital, Sisir Shekhar, Simal in Nepal and L4704, Pusa Vaibhav in India, Alemaya in Ethiopia and Idlib-2, Idlib-3 in Syria have been released having high Zn and Fe content (Thavarajah et al. 2008).

Along with the cereals and legumes some vegetables varieties are also developed as they are the main source of antioxidants in human diet. Some varieties of potato have been obtained by collecting 1000 genotypes that have more antioxidants and Cu, Fe, Mn, and Zn concentrations (Andre et al. 2007). With the collaboration of International potato center (CIP) and HarvestPlus an advanced variety has been developed by crossing diploid Andean landrace potatoes (high Zn and Fe) with tetraploid clones (disease resistant). One more variety INIA 321 Kawsay with high Zn and Fe content in Peru has been developed under National Institute for Agrarian Innovation's (INIA) Potato Program (Andre et al. 2007). Cassava and cauliflower have a wide range of genotype differences particularly for minerals (iron and zinc), so new varieties can be developed by breeding technique (Chavez et al. 2005). Through breeding technology, the biofortified edible crops can be produced having high essential micronutrients (Table 8.3) that will definitely improve the health and economic conditions of the world's population.

8.3.3 Application of Transgenic Method

The application of biotechnology in developing nutrient rich transgenic crops has been started since last 20 years. Genetic engineering is a technique that concerned with the specific genes with desired traits. Once a gene with specific trait has been identified and with the help of marker and promoter genes a new plant with improved nutrient content will be produced by inserting a gene through nonviable virus called *Agrobacterium* as a carrier. GE (Genetic engineering) leads to the development of transgenic crops also known as genetically modified organism (GMOs). When there is less or no genetic variation in nutrients concentration among plant varieties then

Table 8.3 Biofortification of food crop through breeding

Plants	Nutrients	Country/Variety	Reference
Rice	Iron	India, Philippines: IR68144-3B-2-2-3 (improved line) Jalmagna	IRRI Gregorio et al.(2000)
	Zinc	Jalmagna	Gregorio et al. (2000)
	Zinc, iron	Bangladesh: BRRIdhan 62, BRRIdhan 72, BRRIdhan 64	CIAT, HarvestPlus; Garg et al. (2018)
Cassava	Iron	Africa: Cassava clones	Maziya-Dixon et al. (2000)
Potato	Zinc, iron, copper, and manganese	BTD0054-3, BTD0118-5	Haynes et al. (2012)
Wheat	Zinc	India: BHU 1, BHU 3, BHU 5, BHU 6, BHU 17, BHU 18 Pakistan: NR 419, 42, 421, Zincol	Velu et al. (2015)
	Zinc and iron	India: WB2	Chatrath et al. (2018)
	Zinc	India: PBW1Zn	Bhati et al. (2016)
Sorghum	Iron	India: ICSR 14001, ICSH 14002 Hybrids: ICSA 661 × ICSR 196, ICSA 318 × ICSR 94, ICSA 336 × IS 3760	ICAR (2016)
	Iron	Nigeria: 12KNICSV (Deko)-188 12KNICSV-22 (Zabuwa)	ICRISAT, HarvestPlus (2016)
Cow pea	Iron	India: Pant Lobia-1, Pant Lobia-2, Pant Lobia-3, Pant Lobia-4	Singh et al. (2017)
Millets	Iron and zinc (Pearl Millet)	India: Dhanashakti Hybrid ICMH 1201 (Shakti-1201)	Govindaraj (2019)
Lentils	Iron and zinc	Bangladesh: Barimasur-4, Barimasur-5, Barimasur-6, Barimasur-7, Barimasur-8 Nepal: ILL 7723- Khajurah-1, Khajurah-2, Shital, Sisir, Shekhar and Simal India: L4704 and Pusa Vaibhav Ethiopia: Alemaya Syria: Idlib-2 and Idlib-3	Darai et al. (2020)
Beans	High iron and zinc	Rwanda: RWR 2245; RWR 2154; MAC 42; MAC 44; CAB 2; RWV 1129; RWV 3006; RWV 3316; RWV 3317; RWV2887	Jha and Warkentin (2020)

Source: Modified from Garg et al. (2018)

this transgenic approach can be an applicable option for developing biofortified crops (Aung et al. 2013). Incorporation of desired traits includes micronutrient enhancement and bioavailability as well as reduction in the anti-nutrients concentrations (that bind with the nutrient and make them unavailable). Genetic modifications particularly affect the redistribution of micronutrients in plant tissues

and mainly enhance their concentration in edible portions of cash crop. Several studies were done where crops are genetically modified to improve their micronutrient levels particularly for Fe and Zn as they are found to be more deficient than the other micronutrients. In rice plant, genetic modification is done by over expressing iron (II)-nicotianamine transporter OsYSL2 to enhance translocation of Fe in the endosperm (Masuda et al. 2012). The transgenic rice crop showed 4-times higher levels of iron than the conventional one. Mugineic acid acts as a ferric ion chelator, and its production is increased in the transgenic rice crop by the expression of mugineic acid synthase gene (IDS3). This gene is over expressed by expressing the soybean ferritin gene (SoyferH2). The transgenic rice crop is found to be tolerant in iron deficient soil and showed 2.5 times higher concentration of Fe. In Myanmar, about 70% population is found to be Fe deficient so here Aung et al. (2013) have produced a transgenic line of rice by over expressing the nicotianamine synthase gene HvNAS1 (increases the transportation of Fe), the Fe (II)-nicotianamine transporter gene OsYSL2 (enhances transportation of iron in the endosperm), and the Fe storage protein gene SoyferH2 (increases accumulation of iron in the endosperm). The milling step during rice crop processing removes the nutrient-rich outer layers of the embryo that leads to reduction in the concentration of Fe and Zn. In order to solve this issue, a transgenic cultivar of rice (indica) with high yield has been developed by expressing SoyferH2 gene. These new line showed enhancement (2.6 times) in the ferritin level. By using MxIRT1 (iron transporter gene) from apple trees a transgenic rice crop is produced that exhibited 3 time higher Fe and Zn accumulation (Tan et al. 2015). By over expressing nicotianamine synthase (OsNAS2) and soybean ferritin (SferH-1) genes, a rice plant can be developed having high Fe and Zn concentration in the endosperm (Trijatmiko et al. 2016). It was reported that in wheat Gpc-B1 (GRAIN PROTEIN CONTENT B1) is quantitative trait locus responsible for increasing the translocation of protein to the grain that consequently increased Fe, Zn, and Mn concentrations in grain (Uauy et al. 2006). Ozturk et al. (2016) have supported positive correlations between protein content and concentration of Fe, Zn, and Mn. Through genetic transformation of Gpc-B1 locus from the wild tetraploid wheat *Triticum turgidum* ssp. *dicoccoides*, the concentration of Zn, Fe, Mn, and protein content can be increased by 10–34% in wheat grain of different recombinant chromosome substitution lines (Distelfeld et al. 2007). For the uptake and translocation of Zn, the most predominant cation transporter families are the members of the ZIP (ZRT, IRT-related protein) and CDF (Cation diffusion facilitator). Some genetically modified varieties of rice with higher level of Zn and Fe, such as IR64 and IR69428, have been produced by over expressing rice ferritin and rice nicotianamine synthase (NAS2) genes at Indian Rice Research Institute (IRRI) from the field trials (Mallikarjuna Swamy et al. 2016). With this technique, several other important staple crops can be transformed to produce biofortified crops (Table 8.4) that have great potential in combating global problem of malnutrition.

Table 8.4 Biofortification of plants by transgenic approaches

Micronutrients and crops	Over expressed genes	Plants	References
Enhancement of Fe storage in rice seeds	OsGluB1proSoyferH1 ^b OsGlb1 proSoyferH1 ^b	<i>Oryza sativa</i> Japonica cv. Kitaake	Qu et al. (2005)
Enhancement of Zn, Mn, and Fe in wheat	Gpc-B1 locus into different recombinant chromosome substitution lines	<i>Triticum turgidum</i> ssp. dicoccoides	Distelfeld et al. (2007)
Enhancement of Fe uptake	Barley IDS3 genome fragment	<i>Oryza sativa</i> Japonica cv. Tsukinohikari	Masuda et al. (2008)
Enhancement of Fe translocation	Ubiquitin pro-OsIRT1	<i>Oryza sativa</i> Japonica cv. Dongjin	Lee and An (2009)
Enhancement of Zn and Fe in rice	over expressing ferritin and nicotianamine synthase (NAS2)	IR64 and IR69428	Zhang et al. (2010)
Fe translocation increased	35S pro-OsIRO2	<i>Oryza sativa</i> Japonica cv. Tsukinohikari	Ogo et al. (2011)
Enhancement of Fe translocation	35S pro- OsNAS1, 2,3	<i>Oryza sativa</i> Japonica cv. Nipponbare	Johnson et al. (2011)
Fe content in endosperm	iron (II)-nicotianamine transporter OsYSL2	<i>Oryza sativa</i>	Masuda et al. (2012)
Transportation of Fe	Nicotianamine synthase gene HvNAS1	<i>Oryza sativa</i>	Aung et al. (2013)
Uptake and root-to-shoot translocation of Zn increased	ZRT/IRT-like protein	barley	Tiong et al. (2015)
Zn, Fe, and Mn content in wheat grain	Gpc-B1 (GRAIN PROTEIN CONTENT B1)	<i>Triticum aestivum</i>	Trijatmiko et al. (2016)
Increased the Fe content	AtIRT1	<i>Oryza sativa</i> Japonica cv. Taipei 309	Boonyaves et al. (2016)
Storage of Fe content increased in endosperm	OsNAS1, HvHAATb	<i>Oryza sativa</i> L. (cv. EYI 105)	Banakar et al. (2017)
Enhancement in Fe concentration	Os DMAS1	<i>Oryza sativa</i> Japonica cv. Dongjin	Bashir et al. (2017)
Uptake of Fe increased	OsYSL9	<i>Oryza sativa</i> Japonica cv. Tsukinohikari	Senoura et al. (2017)
High accumulation of iron and zinc	OsHMA7transcript levels	<i>Oryza sativa</i>	Kappara et al. (2018)

(continued)

Table 8.4 (continued)

Micronutrients and crops	Over expressed genes	Plants	References
Fe content increased	OsYSL1	<i>Oryza sativa</i> Japonica cv. Zhoghua11	Zhang et al. (2018)
Fe content increased in grain	OsNAS2	<i>Triticum aestivum</i> (cv Bob White)	Beasley et al. (2019)
Zn and Fe concentration increased	IRT1 (iron transporter) and FER1 (ferritin) genes	Cassava	Ghislain et al. (2019)

8.4 Conclusions and Future Prospective

The biofortified crops are not easily accepted by the poor farmers due to lack in the awareness about their benefits on human health. Particularly, in developing countries, malnutrition is one of the inevitable problems due to more population. Because of this, the food provided to them is not having sufficient level of micronutrients that not only harms their health but also increases the susceptibility towards various diseases. Consequently, it leads to considerable loss in Gross Domestic Product and shows devastating effects on socio-economic condition of country. To deal with this situation, biofortification of crop varieties is one of the most sustainable and cost-effective approach. By applying different ways of biofortification whether agronomic or genetic, different biofortified crops can be produced that provide nutrients directly to the common people in their natural form. The biofortified crop varieties act as important sources of nutrients to poor people and also provide nutritional security. In order to enhance the acceptance rate of these biofortified crops, the farmers should be participated in the awareness programs that demonstrate their beneficial roles. The biofortified crops improve the health and nutritional status of the young generation. This process can adequately supply the food to those people, who are underprivileged and low-income households. After developing biofortified crops, there are no further charges, so this strategy can be a sustainable way to manage the hidden hunger particularly in developing and under-developed countries.

Acknowledgments We are very grateful to The Head, Department of Botany, Banaras Hindu University, Varanasi for providing necessary facilities. The University Grants Commission, New Delhi is thankfully acknowledged for providing financial assistant to Dr. A. Singh as PI [F.30-431/2018(BSR)].

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