Biofortification of Crop Plants: A Practical Solution to Tackle Elemental Deficiency

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Abstract

Malnutrition englobes overnutrition and undernutrition. One in four children suffer from chronic undernutrition and approximately 820 million people have a caloric deficit. The effects of malnutrition are transgenerational and they have an impact from the individual to the national level. Although globally there is sufficient food for all, several countries have inadequately domestic food production. Moreover, the deficit in micronutrient achieves about 3 billion people worldwide due to the lower levels in food or availability of these micronutrients for absorption by the intestines. Therefore, agronomic sciences have an important role in providing nutritious food (quality) rather than adequate calories (quantity). In this scenario, biofortification is a notable tool to improve individual nutritional status. Biofortification is the use of the most appropriate biotechnological or traditional breeding practices for micronutrient enrichment (such as vitamins and chemical elements) of staple crops. From the chemical elements considered essential, the deficiencies of calcium, copper, iodine, iron, magnesium, selenium, and zinc are the most common. Several studies for biofortification were conducted focusing the use of agronomic approaches (use of fertilizers in soils, irrigation water, and hydroponic cultivation systems, or by the foliar application during plant growth), conventional breeding, and genetic approaches (the ancient breeding to the modern genetic engineering employing synthetic genes), and the plant growth-promoting microorganisms (PGPM) approaches (use of microorganisms in soil/plant rhizosphere during plant growth). These biofortification approaches have disadvantages and advantages and are dependent on important variables such as farming practices and soil properties. Moreover, biofortification must be associated with the plant-resistance to stress during...
cultivation, yield improvement, food color/palatability, and the bioavailability of the nutrients after human ingestion. The highest number of publications on biofortification are from countries that are among the main food producers in the world (the USA, India, China, Australia, and Brazil), evidencing the importance of this technique in contributing to more nutritious food, especially for the poverty population.

**Keywords**

Zinc · Iodine · Breeding · Genetic · Fertilizers · Bioavailability · Hidden hungry · Farming practices

### 7.1 Why Crop Biofortification Is Necessary?

According to the Food and Agriculture Organization of the United Nations (FAO 2019) after continuously declining for over a decade, global hunger is on the rise again, especially in regions where economic slowdowns occurred. It is also worth noting that this tendency would be more pronounced in the next following years as a consequence of the coronavirus (COVID-19) pandemic and its economic impacts (Hafiz et al. 2020).

Malnutrition englobes both undernutrition (wasting, stunting, underweight, and mineral- and vitamin-related malnutrition) and overnutrition (overweight, obesity, and diet-related noncommunicable diseases) (Dukhi 2020). Around 820 million are in a state of caloric deficit (FAO 2019); nearly one in four children suffer from chronic malnutrition; 52 million children suffer from acute malnutrition; and two billion adults are overweight (Meybeck et al. 2018). Malnutrition is a root cause of many health disorders and it is an imbalance of needs and intake of nutrients and calories. The main reasons for this problem are unavailability or lack of access of food; poor diet (due to a person’s inability to select, take-in, and absorb the nutrients in the food); vulnerability of an individual (i.e., increased micronutrient needs during certain life stages, including pregnancy; and health problems such as diseases, infections, or parasites that can spread in unhealthy environments with poor water, sanitation, and hygiene conditions) and finally, decrease in the micronutrient content of common crops due to productivity demands and climate change (Stein 2010; Von Grebmer et al. 2014; Nelson et al. 2018). According to Nelson et al. (2018), the challenge in 2050 will be providing nutritious diets rather than adequate calories and future studies and policies should emphasize nutritional quality by increasing the availability and affordability of nutrient-dense foods and improving dietary diversity (Nelson et al. 2018). However, in the past, the main focus of the professionals from the agriculture field was on yield increasing without balancing the nutritional qualities of staple crops (Stein 2010). Production systems must, therefore, align with nutritional and health goals (Geyik et al. 2020).
Chronic malnutrition effects are transgenerational and they have an impact at the individual, community, and national levels in the short- and long term (Reinhardt and Fanzo 2014). Thus, dietary solutions that could manage to balance nutritional, economic, environmental, and health pillars are a great challenge for a sustainable future and will require the efforts of agriculturists, public health professionals, educators, nutritionists, policy-makers, and food industries (Tilman and Clark 2014).

Minerals and vitamins malnutrition are defined as hidden hunger and its effects hold significant and immediate negative consequences for the cognitive and physical development of children, however in long term may cause profound consequences in health, on productivity and economic potential in later adulthood (Ruel-Bergeron et al. 2015; Biesalski 2013). In diets from nutritionally vulnerable groups, the co-occurrence of deficiencies from more than one micronutrient is common (Ruel-Bergeron et al. 2015). The most affected continents are Africa and South Asia, nevertheless, it occurs globally, especially to underprivileged people (FAO 2019). In a recent study, Geyik et al. (2020) have explored the spatiotemporal trends in dietary nutrient content and adequacy of primary production based on the production of 174 commodities covering a period of 1995–2015 for 177 countries. The authors highlighted that while total production can adequately provide the global human population with all nutrients except vitamin A, more than 120 countries have inadequate domestic production (Geyik et al. 2020).

Mineral nutrients are fundamentally metals and other inorganic compounds (Gupta and Gupta 2014). Adequate mineral intake is needed for the maintenance of normal organism functions. However, about 3 billion people worldwide have a micronutrient deficient diet (Khush et al. 2012). Factors contributing to this scenario are low concentrations or low bioavailability of these nutrients in food (El-Ramady et al. 2014).

Considering the hidden hungry is a serious public health concern, many strategies have been developed to overcome this problem (Khush et al. 2012). No single intervention will offer a “silver bullet” to micronutrient deficiencies, but there are some strategies commonly employed, such as supplementation, dietary diversification, food fortification, and biofortification (Bouis and Saltzman 2017; Khush et al. 2012; White and Broadley 2009). Although been defended by nutritionists, dietary diversification is a contradictory strategy since people tend to return to their old habits (Khush et al. 2012). The World Health Organization (WHO) highlights food fortification and nutrient supplementation as strategies to combat malnutrition (WHO 2019). The Consultative Group on International Agricultural Research (CGIAR) emphasizes the importance of biofortification through breeding and biotechnological approaches (Khush et al. 2012). By making staple foods more nutritious, people can overcome malnutrition without changing their habits.

According to Nestel et al. (2006), the definition of biofortification is the process of development of micronutrient-rich staple crops using the most suitable traditional breeding practices and recent biotechnology to develop staple crops (Nestel et al. 2006). It is important to biofortified staple foods even if they accumulate
micronutrients in a relatively low rate since they are consumed regularly in larger quantities for many vulnerable populations in the way that they can enhance the micronutrient status of these populations (Junqueira-Franco et al. 2018). However, the feasibility of biofortification depends on: (1) nutrients bioavailability for plants and humans; (2) nutrients stability after harvesting of the crop (not degrade during processing, storage, and preparation); (3) the acceptance of the crop sensory qualities by producers and consumers in the target regions; (4) provide high yielding and profitability to the producers (Sharma et al. 2017). This process should be comparatively cost-effective, sustainable, and long-terms of delivering more micronutrients (Saltzman et al. 2013).

There is an estimative that by the end of 2018, 7.6 million farming households were growing biofortified planting material, benefiting around 38 million people (HarvestPlus 2019). According to Herrington et al. (2019), the selection of the regions, crops, and micronutrients to prioritize biofortification should be based on production, consumption, and micronutrient deficiency using country-level data (Herrington et al. 2019). Also, the continuous search for new techniques, or the improvement of the existing biofortification techniques, is essential to continue this positive scenario and expand the food biofortification around the world. In this chapter, an overview of the biofortification of crop plants will be described, and several studies showing a wide variety of biofortification approaches will be discussed to demonstrate the main challenges and trends.

7.2 Mineral Requirements in Human Nutrition

Considering dietary minerals, there are more than twenty elements considered essential for human body maintenance (Williams 2005). Adequate mineral intake is needed for the maintenance of normal organism functions. However, about 3 billion people worldwide have a micronutrient deficient diet (Khush et al. 2012). Factors contributing to this scenario are low concentrations or low bioavailability of these nutrients in food (El-Ramady et al. 2014). The hidden hunger or micronutrient deficiencies resulting from unbalanced diets is a high priority issue that impedes human and economic development (Khush et al. 2012; Valença et al. 2017). Nowadays, the big challenge is increasing the productivity and the concentration of micronutrients in food crops (El-Ramady et al. 2014).

Around the world, the most common and devastating mineral deficiencies involve calcium, copper, iodine, iron, magnesium, selenium, and zinc. The main functions, as well as the problems related to deficiency and/or excess, are presented in Table 7.1 (White and Broadley 2009; Khush et al. 2012). It is worth mentioning that worldwide, starchy food crops such as rice, maize, wheat, cassava, and legumes are the main focus of biofortification programs. It occurs because these foods are prevalent in the diet of the majority world population, especially for the most vulnerable populations who do not have access to supplements, diverse diets, and commercially fortified foods (Saltzman et al. 2013). Studies have shown that some crops such as
Table 7.1 Main functions of minerals in the human organism and some problems related to inadequate intake of calcium, copper, iodine, iron, magnesium, selenium, and zinc in the human diet

| Element | Description |
|---------|-------------|
| Calcium (Ca) | It is the most abundant mineral in the human body, and it is present mainly in the skeleton. It plays many essential functions, such as supporting the structure and hardness of bones and teeth, being also vital for muscle movement, enzymes, hormones release, and blood movement through blood vessels (Weaver 2012; NIH 2020a; Gharibzahedi and Jafari 2017). Besides, nerves need Ca to transmit messages between different parts of the body. The average recommended intake for this element in adults is 1000 mg day\(^{-1}\) (NIH 2020a). In general, Ca ingestion around the world is below the recommended intake, which increases the risk of many diseases (Weaver 2012). Insufficient Ca intake leads the body to take it from bone to keep healthy levels in the blood. Calcium deficiency can cause osteoporosis and fractures due to the decrease in bone mass. Other possible consequences are convulsions, numbness and tingling in the fingers, and abnormal heart rhythm (NIH 2020a). |
| Copper (Cu) | The essentiality of Cu is linked to brain development, maintenance of immune and nervous systems, and gene activation (NIH 2020b; Gharibzahedi and Jafari 2017). Copper is a constituent of various enzymes, which take part in many metabolic reactions. These cuproenzymes are involved in energy production and utilization, synthesis of proteins of blood vessels, and connective tissues (NIH 2020b). The recommended intake of Cu for adults is 900 μg day\(^{-1}\) (IOM 2000; NIH 2020b). Some effects of Cu deficiency are extreme tiredness, high cholesterol levels, weak and brittle bones. Connective tissue disorders, loss of balance, and coordination can also occur. People with a diet deficient in Cu are at increased risk of infection (NIH 2020b). |
| Iodine (I) | It is an essential mineral for thyroid function, being constituent of the hormones T3 and T4 (Gonzali et al. 2017). These hormones are relevant for the body’s metabolism, growth, development, reproduction, nerve and muscle function, production of blood cells, among others (Gharibzahedi and Jafari 2017). During infancy and pregnancy, thyroid hormones are essential for proper brain and bone development (IOM 2000; NIH 2019a). The recommended intake of I is 150 μg day\(^{-1}\) for adults (NIH 2019a). Iodine deficiency is a widespread problem, affecting both developing and developed countries (Gonzali et al. 2017). In children, cognitive development and mental health can be compromised. Among pregnant women, some possible consequences are spontaneous abortion, stillbirth, and congenital abnormalities (NIH 2019a). According to the World Health Organization (WHO), iodine deficiency is the most prevalent cause of brain damage in the world (WHO 2013). |
| Iron (Fe) | It is responsible for oxygen transport, antioxidant activity, hormone synthesis, neurodevelopment, connective tissues synthesis, and energy metabolism (Aggett 2012; Gharibzahedi and Jafari 2017). Iron deficiency is the most common and widespread nutritional disorder in the world, leading to severe anemia. It is estimated that iron deficiency anemia (IDA) affects 2 billion people around the world, mainly in developing countries. Meantime, it is the only nutrient deficiency that is also prevalent in industrialized countries (WHO 2019). The recommended intake of Fe varies between 8 and 18 mg day\(^{-1}\) for adults, depending on gender (NIH 2020c). Among the consequences of IDA in adults are weakness, irritability, and reduced work productivity. In children, IDA can lead to susceptibility to disease, impaired physical and mental development, and increased mortality risk. In developing countries, IDA affects around 40% of preschool children (Khush et al. 2012; WHO 2019). |
Table 7.1  (continued)

| Element   | Description                                                                 |
|-----------|-----------------------------------------------------------------------------|
| Magnesium (Mg) | It is the fourth most abundant cation in the organism and is needed for over 300 metabolic reactions (Volpe 2012). It takes part in the synthesis of DNA, protein, and bone (Gharibzahedi and Jafari 2017). Beyond that, it is important for the regulation of blood sugar levels, blood pressure, muscle, and nerve function. The recommended intake of Mg varies between 310 and 420 mg day$^{-1}$ for adults, depending on gender (NIH 2019b). Magnesium deficiency leads to nausea, vomiting, loss of appetite, weakness, and fatigue. In extreme cases, the symptoms can include personality changes, seizures, abnormal heart rhythm, and muscle cramps (NIH 2019b). Hypertension, cardiovascular disease, and type 2 diabetes mellitus can also be related to Mg deficiency (Volpe 2012). |
| Selenium (Se) | It is an essential trace element in the human diet, being necessary for a narrow concentration range (Skalickova et al. 2017; Jones et al. 2017). The Recommended Dietary Allowance (RDA) for Se in adults is 55 μg day$^{-1}$, while the tolerable upper intake level (UL) is set at 400 μg day$^{-1}$ (IOM 2000). It plays an essential role in the formation of selenoproteins and selenoenzymes, which are very important due to its antioxidant properties aiding on the body protection from the damaging effects of free radicals, potentially toxic elements (e.g., mercury), and other dangerous substances (Skalickova et al. 2017; Yu et al. 2005; Gharibzahedi and Jafari 2017). It is also involved in several metabolic processes such as the production and regulation of thyroid hormones, regulation of redox status, increasing the resistance of the immune system, and reducing risks of some chronic diseases (Skalickova et al. 2017; IOM 2000). Approximately one in seven people around the world have a low Se intake (Jones et al. 2017). Selenium availability in the diet is controlled by some factors, namely geographical location, soil concentration, interactions in the soil-plant system, seasonal changes, and food processing (Jones et al. 2017; Navarro-Alarcon and Cabrera-Vique 2008). Selenium deficiency leads to several metabolic disorders. Some possible effects of inadequate Se intake are Keshan disease (human cardiomyopathy in Se deficient children) and Kashin–Beck disease (human cartilage disease). Selenium deficiency may also increase predisposition to other illnesses (IOM 2000). |
| Zinc (Zn) | It has functions related to growth, physical and cognitive development, and immune function. It is essential for genetic expression, cell division, and programmed cell death. Also, it plays a vital role in the function of many enzymes, such as copper-zinc superoxide dismutase, alcohol dehydrogenase, and other enzymes in the nervous system (Mafra and Cozzolino 2004; Gharibzahedi and Jafari 2017). The recommended intake of Zn varies between 8 and 12 mg day$^{-1}$ for adults, depending on gender (NIH 2020d). Approximately one-third of the world’s population has a zinc-deficient diet. Zinc deficiency can harm the immune system, beyond causing oxidative damage (Mafra and Cozzolino 2004; Khush et al. 2012). Other consequences include neuropsychological impairment, hypoguesia, hypogonadism and dermatitis (Mafra and Cozzolino 2004). |
rice, cassava, and maize, in general, present more effective results when submitted to biofortification (Díaz-Gómez et al. 2017).

### 7.3 Biofortification Approaches

Biofortification approaches are usually used to increase the bioavailable mineral content of food crops, and some techniques have been developed and applied for this purpose (White and Broadley 2009; Khush et al. 2012). Despite this, biofortification techniques can be employed with different goals. Crops production in mineral-deficient soils may compromise the growth and yield, for example, and biofortification is also useful to solve these issues (Chugh and Dhaliwal 2013). This fact is because these elements are also essential for the proper development of plants. Thus, biofortification strategies have been also studied aiming yield improvement, resistance to stress, and food palatability (Valença et al. 2017; Gonzali et al. 2017; White and Broadley 2009; Navarro-Alarcon and Cabrera-Vique 2008).

It is important to note that there is a path followed by the minerals from the soil to the human body, passing through the crop and the food, and biofortification strategies should be carefully selected considering each application (Valença et al. 2017). In this path, many factors can influence elemental bioavailability, as shown in Fig. 7.1. In this way, some challenges must be overcome to be successful in biofortification (Valença et al. 2017; White and Broadley 2009). The first challenge is related to the presence and bioavailability of elements in the soil (Valença et al. 2017). It is necessary to be aware of the chemical forms of elements that plant roots can acquire, for example. The biological and physicochemical properties of the soil influence the chemical forms of the elements that will be present in the rhizosphere solution. In this way, the phytoavailability may be affected, limiting the accumulation of these species by crops (White and Broadley 2009). Another critical issue is that different plant varieties can accumulate mineral elements in a wide concentration range, therefore the crop variety needs to be carefully selected for effective
biofortification (White and Broadley 2009; Valença et al. 2017). After absorbed by roots, nutrients are translocated to the edible tissues of the crop. Some factors that can influence this process are crop variety and processing methods. Finally, the human ability to absorb nutrients is influenced by individuals’ health, dietary intake, and cooking methods (Valença et al. 2017).

Although fertilizers are often applied when the soil is deficient in mineral elements, there are biofortification strategies based on increasing element uptake from soils. These techniques focus on improving the uptake of nutrients by the roots and their redistribution to edible tissues (White and Broadley 2009; Durán et al. 2013). On the other hand, there are agronomic approaches based on fertilizer application in leaves, seeds, as well as in irrigation water and hydroponic cultivation systems. These strategies emerged to circumvent the limitations related to the complex reactions of minerals in the soil and enhance the plant biofortification process. Some minerals have low mobility in the soil depending on the chemical conditions of the soil (pH, composition, etc.) and end up becoming unavailable to plants. In brief, several processes have been used to promote the biofortification of crop plants such as conventional and mutational breeding, genetic engineering, agronomic approaches, among others (Garg et al. 2018; Bouis and Welch 2010; Hirschi 2009; Saltzman et al. 2013). In this chapter, biofortification strategies will be classified into three categories: agronomic, conventional breeding and genetic, and plant growth-promoting microorganisms (PGPM) approaches.

It is worth mentioning that the development of studies to evaluate the strategies for the biofortification of foods has grown significantly in the last 20 years, and approximately 1918 documents were published from January 2000 to March 2020 as can be seen in Fig. 7.2. By the end of March 2020, more than 100 documents had already been published, which shows that this upward trend is expected to continue given the relevance of crop plant biofortification today.

Regarding the percentage of publications by country or territory, the USA (15%), India (10%), China (6%), Australia (5%), and Brazil (4%), stand out as they account for about 40% of the publications presented in Fig. 7.2. Indeed, these countries with a higher number of publications on crop plant biofortification, have a large fertile territory and are the main food producers in the world. In Fig. 7.3, a choropleth map showing the percentage of publications for the biofortification of foods by country or territory is presented.

7.3.1 Agronomic Approaches

The agronomic approaches are based on the application of chemical substances containing minerals (fertilizers) during plant growth aiming to increase micronutrient concentrations in edible tissues (Valença et al. 2017; White and Broadley 2009). The most common agronomic approach used for crop plant biofortification is the...
application of fertilizers in the soil. Solutions of inorganic salts are the most widespread for this purpose. However, micronutrients delivered by using these solutions usually have relatively low availability in the soil since they may be fixed as insoluble forms, or still, be easily released and leached down the soil profile.
(El-Ramady et al. 2014). On the other hand, the soil application of algal-based iodized organic fertilizer has proved to be an interesting choice for I biofortification in crop plants (Weng et al. 2013, 2008b; Hong et al. 2008, 2009). Also, some studies have shown that the application of organic amendments such as biosolids biochar and hyperaccumulator plants can also be efficient and advantageous for Fe, Se, and Zn biofortification in crop plants (Gartler et al. 2013; Bañuelos et al. 2015; Ramzani et al. 2016). Nevertheless, the efficiency of soil fertilizer application is dependent on several factors, especially those related to management practices and soil factors, which affect the mobility of elements in soil and their bioavailability for plants.

In this way, the natural process in which plants absorb nutrients through the leaves has been extensively explored in agriculture for crop plant biofortification by foliar application of fertilizers. The foliar application consists of the foliar spray or application of nutrients on aboveground plant parts to supply traditional soil applications of fertilizers. It may be considered one of the most important approaches used for delivering nutrients in suitable concentrations to plants, improving their nutritional status, the crop yield as well as their quality (Alshaal and El-Ramady 2017). This type of application is much less influenced by external factors than soil fertilizer application and, therefore, it has been the target of several studies aiming at the biofortification of crop plants. The use of nanoparticles containing elements that are intended to increase the concentration in the crop plants should be emphasized among the fertilizers used for foliar application. Recent studies have shown that, in addition to biofortification with essential elements, nanoparticles application has promoted the mitigation of toxic elements, such as Cd and Pb, present in the cultivation soil (Hussain et al. 2018, 2020).

Other strategies that have also been successfully used for crop plant biofortification are the application of fertilizers in irrigation water, hydroponic systems, and seeds before cultivation (De Figueiredo et al. 2017; Smoleń et al. 2014, 2015, 2018; Smoleń and Sady 2012; Trolove et al. 2018; Rizwan et al. 2019). In general, agronomic biofortification is simpler and less expensive in the short term when compared with genetic approaches. On the other hand, fertilizer application must be done regularly and may cause damages to the environment, beyond increasing labor and cost in the long term. Some studies showing the application of common agronomic strategies are shown in Table 7.2. In short, these studies demonstrate the main challenges and trends of agronomic approaches for crop plant biofortification.

There is scientific evidence that the nutritional quality of staple crops can be improved by using agronomic biofortification. Valença et al. (2017) stated that these techniques are useful tools for enhancing micronutrient content in edible parts of food crops. Some factors that can influence the success of these approaches are the soil composition, application method, plant species, which can affect mineral mobility and accumulation, and the nutrient accumulation on plant tissues. Thus, some strategies may be limited by geographical locations and crop types, so they may not be applied universally. The efficiency of micronutrient fertilization can be optimized by using integrated soil fertility management, such as combination with organic and NPK fertilizers and selection of improved crop varieties, which can more effectively capture nutrients and accumulate them in consumed parts (Valença et al. 2017).
### Table 7.2  Studies demonstrating the most common agronomic approaches used for crop plant biofortification

| Application | Crop plants | Elements | Results                                                                                                                                                                                                 | Reference                          |
|-------------|-------------|----------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------|
| Foliar      | Lettuce     | Se       | Effects of Se biofortification of lettuce was studied on the toxicity and transfer of Hg from soil contaminated with HgCl₂ to the terrestrial food chain, with slug as a primary consumer. Selenium foliar application increased Se concentration in the lettuce and did not affect Hg bioaccumulation. Besides, Se application increased Hg bioavailability for slugs. | Kavčič et al. (2020)               |
|             | Potato      | Se       | The foliar application of Na₂SeO₄ and Na₂SeO₃ for Se biofortification of potato was assessed. Selenate was more efficient than Na₂SeO₃ for Se accumulation, and the highest tuber Se concentration was obtained at the tuber bulking stage. Moreover, the major species in tubers treated with inorganic Se was selenomethionine (up to 80% of total Se) suggesting the foliar application is appropriate for the production of Se-rich potatoes. | Zhang et al. (2019)                |
| Rice        | Se          |          | Nanoparticles of Se and Si were evaluated for plant growth, metals accumulation, and Se biofortification of rice. The concentrations of Cd and Pb in brown rice were significantly decreased, and the combined application of Se and Si nanoparticles | Hussain et al. (2020)             |

(continued)
Table 7.2  (continued)

| Application | Crop plants | Elements       | Results                                                                 | Reference                      |
|-------------|-------------|----------------|-------------------------------------------------------------------------|--------------------------------|
| Rice        | Se and Zn   | The authors found significant differences in Se and Zn biofortification capability of two Mozambican rice cultivars evaluated in this study. The genotype labeled IR-87684-23-2-3-2 responded better to biofortification. A preferential Se and Zn accumulation in the outer part of the grain was also observed. Selenium and zinc concentrations in whole grain (IR genotype) ranged from 12.6 (control) to 17.9 mg kg\(^{-1}\) and from 0.0 (control) to 6.1 mg kg\(^{-1}\), respectively. | Mangueze et al. (2018)          |
| Wheat       | B, Cu, Fe, Mn and Zn | The foliar application was useful for growth and yield parameters. Besides, foliar application of micronutrients at tillering, jointing, and booting stages allowed the enriching of wheat grains with B, Cu, Fe, Mn, and Zn, with an uptake up to 48% (about 12 g ha\(^{-1}\)), 74% (38 g ha\(^{-1}\)), 44% | Aziz et al. (2019)              |
Table 7.2 (continued)

| Application | Crop plants | Elements | Results | Reference |
|-------------|-------------|----------|---------|-----------|
| Wheat       | Se          |          | The foliar spray was effective for Se biofortification of wheat. The dosage of 21 g ha\(^{-1}\) of Se was the most suitable (Se absorption efficiency by wheat grains was of about 3%). | Lara et al. (2019) |
| Foliar and hydroponic system | Lettuce | I and Se | Iodine and Se foliar application resulted in better results (higher I and Se concentration in lettuce) in comparison with the hydroponic system application. Iodine and Se concentrations were up to about 75 and 780 mg kg\(^{-1}\) in leaves, and up to 85 and 95 mg kg\(^{-1}\) in roots, respectively. | Smoleń et al. (2014) |
| Foliar, hydroponic system and soil | Nectarine, plum, potato, and tomato | I | For soil and foliar I application, the highest I concentration was 0.95 and 1.43 μg kg\(^{-1}\) for plum and nectarine, respectively. For potato and tomato, the highest I concentration was of 8.94 and 14.4 μg kg\(^{-1}\), respectively. Nectarine and plum trees accumulated lower I concentration in their edible tissues compared with potato and tomato. Besides, hydroponic culture proved to be the most efficient system for I biofortification of tomato, since accumulated up to 242 μg kg\(^{-1}\) fresh fruit. Iodine was preferably stored in the leaves. | Caffagni et al. (2012) |
Table 7.2 (continued)

| Application       | Crop plants       | Elements | Results                                                                                           | Reference             |
|-------------------|-------------------|----------|---------------------------------------------------------------------------------------------------|-----------------------|
| Only a small I fraction was transported to plum tree branches and fruit, as well as to potato stems and tubers. | Tomato | I        | Tomato plants were treated with radioactive iodine (Na\(^{125}\)I), and the results indicate that tomato is an excellent crop for I biofortification. Iodine was taken up better when supplied to the roots using hydroponically grown plants. Nevertheless, a considerable I concentration was also stored after foliar application, which suggests that I is also transported through the phloem. Besides, according to the authors, tomato plants can tolerate higher I concentrations. Iodine is stored both in the vegetative tissues and fruits in concentrations that are more than sufficient for the human diet. | Landini et al. (2011) |
| Foliar and soil   | Kohlrabi, lettuce, and radish | I        | KIO\(_3\) and KI solutions (concentrations of up to 15 kg ha\(^{-1}\)) were evaluated for I biofortification of kohlrabi, lettuce, and radish. Iodine concentration in the edible plant parts increased with the addition of the I fertilizer application. Better results (higher I accumulation and lower growth impairment) | Lawson et al. (2015)   |
Table 7.2 (continued)

| Application       | Crop plants | Elements | Results                                                                 | Reference                      |
|-------------------|-------------|----------|------------------------------------------------------------------------|--------------------------------|
| Maize and wheat   | Zn          | Foliar and soil application was performed using ZnSO$_4$.7H$_2$O. Zinc concentration in grain increased up to 37% for maize and 89% for wheat through foliar Zn application. Besides, foliar Zn application increased up to 22% Fe concentration in maize grain. Soil Zn application does not affect the Zn concentration in both grains. | Wang et al. (2012) |
| Pea               | Zn          | Foliar Zn application in combination with soil Zn application promoted increases in grain Zn concentration up to threefold. The effect of processing (freezing and cooking) was also studied in fortified grains and a decrease of about 30% in grain Zn concentration was observed. The combination of soil and foliar application could be a good option for biofortifying field peas. | Poblaciones and Rengel (2016) |
| Rice              | Zn          | Zinc concentration increased by 25% and 32% by foliar (about 32 mg kg$^{-1}$) and foliar + soil (about 35 mg kg$^{-1}$) Zn applications, respectively, and only 2.4% by soil (about 19 mg kg$^{-1}$) Zn application. | Phattarakul et al. (2012) |
| Wheat             | Zn          | Nanoparticles of ZnO were studied as an alternative for Zn biofortification and | Hussain et al. (2018) |

(continued)
| Application          | Crop plants | Elements | Results                                                                                                                                                                                                 | Reference                                      |
|----------------------|-------------|----------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------|
| Hydroponic system    | Beans       | Se and Zn | Biofortification with Se and Zn did not affect the Fe bioavailability, and it proved to be an interesting alternative to increase the food quality of beans. While Se and Zn concentration had an increase of up to threefold twofold, respectively, when compared to those observed for control. | De Figueiredo et al. (2017)                     |
| Chinese cabbage      | I           | NaI and NaIO$_3$ solutions containing I concentrations of up to 5.0 mg L$^{-1}$ and an organic iodine fertilizer (seaweed composite) were evaluated for I biofortification of lettuce. Results show that I uptake by cabbage was more effective using NaIO$_3$ when low I concentration (<0.5 mg L$^{-1}$) was applied. On the other hand, I uptake was also useful using NaI when I concentration of 0.5 mg L$^{-1}$ or higher was applied. NaI and NaIO$_3$ provided a quicker supply for | Weng et al. (2008a)                            |
### Table 7.2 (continued)

| Application | Crop plants | Elements | Results | Reference |
|-------------|-------------|----------|---------|-----------|
| cabbage uptake, but higher I concentrations were toxic to plant growth. In short, the seaweed composite provided a more sustainable I biofortification for cabbage. | | | | |
| Lettuce | I | KI and KIO\(_3\) solutions containing I concentrations of up to 240 μM were evaluated for I biofortification of lettuce. Based on the results, I concentrations of up to 40 μM using KI were the most appropriate because these concentrations did not reduce biomass when compared to control plants. Also, in these conditions, it was observed the highest foliar I accumulation, and the treated plants show a significant increase in antioxidant compounds. | | Blasco et al. (2008) |
| Lettuce | I | I concentrations of up to 129 μg L\(^{-1}\), applied as iodate (IO\(_3^-\)) or iodide (I\(^-\)), was evaluated for I biofortification of lettuce in a winter and summer trial. I application did not affect plant biomass, produce quality, or water uptake. Nevertheless, increases in I concentration significantly enhanced I biofortification of the plant, and I concentrations in plant tissue were up to fivefold higher with I\(^-\) application. The outer | | Voogt et al. (2010) |
Table 7.2  (continued)

| Application | Crop plants | Elements | Results | Reference |
|-------------|-------------|----------|---------|-----------|
|             |             | leaves presented the higher I concentrations. The highest I concentration rates in both trials resulted in a total I concentration of 653 and 764 μg kg$^{-1}$ leaf fresh weight. |         |           |
| Lettuce     | I           |          | The effect of 5-iodosalicylic acid (5I-SA) on the growth, chemical composition, and efficiency of iodine biofortification of lettuce was evaluated. A strong toxic effect on lettuce was observed only when the highest I concentrations as 5I-SA (40 μmol L$^{-1}$) were applied. Iodine concentration of up to 8 mmol L$^{-1}$ as 5I-SA resulted in higher I transfer factor values than those obtained after the application of KIO$_3$ or KI0$_3$ plus 5I-AS. | Smoleni et al. (2017) |
| Pepper      | I           |          | The I hydroponic system application using 0.25–5.0 mg L$^{-1}$ KI solutions made it possible to obtain concentrations of up to 1330 μg kg$^{-1}$ fresh weight, matching the World Health Organization recommendations about dietary iodine allowance. Besides, low–moderate levels (0.25–1.0 mg L$^{-1}$) improved the fruit quality, which demonstrates that pepper can be used as a candidate crop for iodine biofortification. | Li et al. (2017) |
Table 7.2 (continued)

| Application | Crop plants | Elements | Results                                                                                                                                                                                                 | Reference                      |
|-------------|-------------|----------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------|
| Potato      | I and Se    |          | The influence of salicylic acid on I and Se biofortification of potato plants was evaluated in this study. The evaluated treatments had no significant effect on the yield of tubers. The simultaneous application of I, Se, and salicylic acid caused a significant increase in the content of Se in the roots but had no significant effect on the content of Se in the tubers. Besides, this application promoted a decrease of Se concentration in the leaves and petioles. | Smolení et al. (2018)          |
| Rice        | I           | KI and KIO₃ solutions (concentrations ranging from 1 to 100 μmol L⁻¹) were evaluated for I biofortification of rice. However, the authors highlight that none of the treatments provided I enough in the edible parts of rice plants to meet the human dietary requirement. Results suggesting differences in uptake or translocation between I forms since KIO₃ treatments had more I partitioning to the roots (56%) on average than did the KI treatments (36%). | Mackowiak and Grossl (1999)    |
| Spinach     | I           | KI and KIO₃ solutions (concentrations of up to 100 μmol L⁻¹) were evaluated for I biofortification of spinach. The solution-to-spinach leaf transfer factors for plants treated | Zhu et al. (2003)              |

(continued)
Table 7.2 (continued)

| Application                  | Crop plants | Elements | Results                                                                                                                                                                                                                                                                                                                                 | Reference                |
|------------------------------|-------------|----------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------|
| Tomato                       | I           |          | The influence of salicylic acid on I biofortification of tomato using KI or KIO₃ was investigated. Salicylic acid combined with KI or KIO₃ increased by about 37% and 157%, respectively, the I accumulation in fruits. Although fruits of plants treated with KI contained significantly more I, the treatment with KIO₃ was the best for nutritional value. | Smoleń et al. (2015)     |
| Irrigation water and seed    | Spinach     | I        | KIO₃ and KI solutions were applied for I biofortification using two treatments: pre-sowing fertilization and fertigation. The latter proved to be much more effective for I biofortification of spinach (up to 339% and 498% for KI and KIO₃, respectively). Also, I biofortification decreased Na and Zn concentration in                                                                                           | Smoleń and Sady (2012)   |

(continued)
### Table 7.2 (continued)

| Application | Crop plants | Elements | Results | Reference |
|-------------|-------------|----------|---------|-----------|
| Seed        | Broccoli, green radish and purple radish sprouts | Se       | The effect of Se addition on the nutrient composition of broccoli, purple radish and green radish sprouts was investigated for Zn biofortification. In short, Se concentration in sprouts increased exponentially with time, when high Se supply was applied (up to 9 μmol Se g⁻¹ seed). | Trolove et al. (2018) |
| Cucumber, lettuce, and tomato | Se | The Se biofortification of cucumber, lettuce, and tomato, based on the use of Se-enriched peat during the pre-transplanting stage was evaluated. The proposed method using Se concentrations of up to 20 mg kg⁻¹ of dry peat induced a Se-enrichment in transplants without adverse effects on plant growth. Edible parts of Se-enriched plants at the end of cropping cycle showed Se concentrations ranging from 29–48 μg kg⁻¹ for cucumber, 23–53 μg kg⁻¹ for lettuce, and 15–20 μg kg⁻¹ for tomato, which was up to 3.4-fold, 8.5-fold, and 1.6-fold, respectively, higher than the control. | Businelli et al. (2015) |
| Application | Crop plants | Elements | Results | Reference |
|-------------|-------------|----------|---------|-----------|
| Wheat | Fe and Zn | Nanoparticles of Fe and ZnO were evaluated for Fe and Se biofortification as well as on the plant growth and Cd mitigation of wheat. When the higher nanoparticle treatments were applied, Cd concentration in the grains was below the threshold level of Cd for cereals (0.2 mg kg\(^{-1}\)). The application of ZnO and Fe nanoparticles increased the Zn and Fe concentrations in roots, shoots, and grains. | Rizwan et al. (2019) |
| Wheat | Zn | Nanoparticles of ZnO were studied as an alternative for Zn biofortification of wheat, and its effects were compared to the ZnSO\(_4\) application. Nanoparticles of ZnO were more effective than the ZnSO\(_4\) application for Zn biofortification of wheat grains but less effective at increasing leaf Zn. At moderate concentrations of ZnO NPs and ZnSO\(_4\), the grain yield and biomass significantly increased. At high concentrations, ZnSO\(_4\) was more toxic than ZnO NPs. | Du et al. (2019) |
| Soil | Aubergine, cucumber, and radish | I | Iodine soil application was performed using mixtures of granular kelp and diatomite with different iodine concentrations (prepared by varying the kelp/diatomite ratio). Iodine concentrations in both leaf and fruit/rhizome tissues | Weng et al. (2008b) |

(continued)
Table 7.2 (continued)

| Application                          | Crop plants                           | Elements | Results                                                                 | Reference          |
|--------------------------------------|----------------------------------------|----------|-------------------------------------------------------------------------|---------------------|
|                                      |                                        |          | increased with the increase of I concentrations in iodized fertilizer applied. The use of diatomite helped to increase the durability of the iodized fertilizer. The organic mixture is a safe and interesting alternative for I biofortification of aubergine, cucumber, and radish. |                     |
|                                      | Barley, maize, potato, tomato, and wheat | I        | KIO₃ and KI solutions (concentrations of up to 23 mmol L⁻¹ and 6 mmol L⁻¹, respectively) were evaluated for I biofortification of barley, maize, potato, tomato, and wheat. The plants tolerated higher I concentrations as IO₃⁻ than I⁻ in the root environment, and barley showed the lowest biomass reductions. In turn, maize showed the most significant biomass decrease due to I toxicity. In all cases, the KI application provides much higher accumulation efficiency than the KIO₃ application. | Caffagni et al. (2011) |
|                                      | Beetroot, broccoli, carrot, corn, courgette, leek, lettuce, onion, radish, spinach, and tomato | Zn       | The efficiency of a biosolids/biochar soil amendment in Zn biofortification of vegetables was evaluated. The biomass and Zn concentration of most species significantly increased using the biosolids and biosolids + biochar treatments. The highest increase in Zn | Gartler et al. (2013) |
Table 7.2  (continued)

| Application                              | Crop plants                  | Elements | Results                                                                 | Reference          |
|------------------------------------------|-------------------------------|----------|-------------------------------------------------------------------------|--------------------|
|                                          |                               |          | concentrations was observed for beetroot, which was of up to 178 and 1200 mg kg\(^{-1}\) in the bulbs and leaves, respectively. Based on results, the mixture of biosolids and biochar is an efficient approach for Zn biofortification of crops with edible leaves as well as beetroot. |                     |
| Bitter tomato, African eggplant, and Turkey berry | K                             |          | The potassium fertilizer application was assessed for the biofortification of vegetables. While the yield of turkey berry was significantly affected by type, rate, and interactive effect of type and rate of fertilizer application, bitter tomato, and African eggplant were affected only by the rate of fertilizer application. Potassium concentrations in leaves were higher than in fruits of all the vegetables, and the highest K concentrations for bitter tomato (2130 mg kg\(^{-1}\) dry weight) and turkey berry (1883 mg kg\(^{-1}\) dry weight) was observed when a KCl solution was used. However, the highest K concentration for African eggplant (1801 mg kg\(^{-1}\) dry weight) was obtained using a sulfate of Potash solution. | Adu et al. (2018)    |
| Broccoli, and carrots                    | Se                            |          | Soils amended with ground shoots of the Se-hyperaccumulator             | Bañuelos et al. (2015) |
| Application | Crop plants | Elements | Results | Reference |
|-------------|-------------|----------|---------|-----------|
| Cabbage, coriander, hot pepper, long cowpea, eggplant, potherb mustard, Chinese cabbage, tomato, cucumber, and spinach | I | A novel approach for I biofortification of vegetables with algal-based iodized organic fertilizer was proposed. Ten species of vegetables were tested, and, in general, the I absorption in these vegetables increased with the increasing amount of the algal-based iodized organic fertilizer used. Besides, I uptake by leaf vegetables was significantly higher than that of fruit vegetables. Iodine concentration decreased from root, leaf, stalk, to fruit. | Weng et al. (2013) |
| Carrot | I and Se | I and Se soil application did not affect yield, but the plants of all genotypes evaluated in this study accumulated both elements in leaves and roots. The concentration of I and Se in roots increased about eightfold and fivefold, respectively. | Smoleń et al. (2019) |
| Carrot, celery, onion, pak choi, spinach, and water spinach | I | KIO₃ was used as fertilizer to evaluate I biofortification of six vegetables. Iodine soil | Dai et al. (2004) |
Table 7.2  (continued)

| Application                  | Crop plants          | Elements | Results                                                                                                                                                                                                 | Reference            |
|------------------------------|----------------------|----------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------|
| Celery, pak choi, pepper, and radish | I                    | Iodine   | Iodine soil application was evaluated for I biofortification using inorganic (KI) and organic (seaweed fertilizer) forms. The I concentrations in soil decreased with time and with plant growth as well. Iodine from KI and seaweed decreased by 50% and 60% of the applied dose, respectively. Iodine accumulation in the edible portion was ranked as follows: pak choi > celery > radish > pepper. Seaweed fertilizer proved to be a better choice for I biofortification of celery, pak choi, pepper, and radish. | Hong et al. (2009)    |
| Chinese cabbage, lettuce, tomato, and carrot | I                    | Iodine   | Both inorganic iodine (KI) and organic seaweed iodine, were evaluated for I biofortification of                                                                                                                  | Hong et al. (2008)    |

(continued)
| Application | Crop plants | Elements | Results | Reference |
|-------------|-------------|----------|---------|-----------|
|             | cabbage, lettuce, tomato, and carrot. Iodine concentrations in vegetables increased with both I application. Iodine accumulation in the edible portion was ranked as follows: cabbage > lettuce > carrot > tomato. Iodine concentration in cabbage was twofold and fourfold higher than that of lettuce and carrot, respectively, and 20-fold higher than that of tomato. In turn, I distribution in vegetable tissues was: root > leaf > stem > fruit, except for carrot. Organic seaweed iodine application demonstrates more potential for durability than KI. | Zn | Zn concentrations reached 42 mg kg\(^{-1}\) (red clay) and 45 mg kg\(^{-1}\) (sandy) in cowpea with Zn soil application against grain Zn concentrations of 36 mg kg\(^{-1}\) and 31 mg kg\(^{-1}\) measured in cowpea grown with no Zn soil application on red clay and sandy soils, respectively. In general, Zn soil application under integrated soil fertility management increased grain yield and grain Zn content. | Manzeke et al. (2017) |
| Cowpea | I | The I soil application using the two lower concentrations (0.10 and 0.25 mg L\(^{-1}\)) stimulated the growth of both plants. I concentration in | Dobosy et al. (2020) |

Green beans, and lettuce |

Dobosy et al. (2020) |
| Application | Crop plants | Elements | Results | Reference |
|-------------|-------------|----------|---------|-----------|
| edible parts of green bean and lettuce was up to 0.6 and 5.2 mg kg\(^{-1}\) dry weight, respectively. | Lettuce | Se | Sodium selenate and selenite were evaluated for Se biofortification of lettuce. Results indicated that Na\(_2\)SeO\(_4\) was less toxic form, and it induced greater biomass, higher Se accumulation, and more antioxidant compounds than did Na\(_2\)SeO\(_3\) application. Sodium selenate concentration of 40 μmol L\(^{-1}\) proved the most suitable for lettuce plants. | Ríos et al. (2008) |
| Onion | Zn | Zn soil application using Zn chelated by EDTA and/or DTPA promoted the better results (up to 7.80 mg kg\(^{-1}\) of total Zn concentration; up to 5.16 mg kg\(^{-1}\) of soluble Zn concentration, and the highest plant biomass and chlorophyll and carotenoid contents). | Almendros et al. (2015) |
| Spinach | I | KI and KIO\(_3\) solutions containing I concentrations of up to 2 mg kg\(^{-1}\) were evaluated for I biofortification of spinach. Biomass productions were not significantly affected, while I concentrations increased with the increasing addition of KI and KIO\(_3\). Potassium iodate application provides much higher I concentrations in tissue plants than KI application. Moreover, | Dai et al. (2006) |
| Application | Crop plants | Elements | Results | Reference |
|-------------|-------------|----------|---------|-----------|
| Tomato      | I           | Several substances containing I (KI and KIO₃, as well as of organic iodine compounds—5-ISA (5-iodosalicylic acid), 3,5-diISA (3,5-diiodosalicylic acid), 2-IBeA (2-iodobenzoic acid), 4-IBeA (4-iodobenzoic acid) and 2,3,5-triIBeA (2,3,5-triiodobenzoic acid)) were applied to evaluate its uptake by tomato plants. Only 2,3,5-triIBeA harmed plant development. Also, 2-IBeA and 4-IBeA were the most active compounds for transferring iodine to fruits and leaves, respectively. | Halka et al. (2019) |
| Tomato      | I           | KIO₃ and KI solutions (concentrations of up to 10 mmol L⁻¹) were evaluated for I biofortification of tomato. Both treatments promoted a significant increase in the I concentration in the fruits that did not affect plant growth and development. Besides, I soil application did not affect fruit appearance and quality, even with the highest concentrations applied. | Kiferle et al. (2013) |
The elements most targeted for crop plant biofortification are micronutrients, such as I, Se, and Zn. This may be associated with the fact that these elements are probably more efficiently absorbed by plants. Moreover, they are extremely important for the human organism and are usually found in very low concentrations in crop

| Application | Crop plants | Elements | Results | Reference |
|-------------|-------------|----------|---------|-----------|
| Wheat       | Fe          | Fe (as sulfate) soil application combined with biochar and S promoted the highest Fe concentration in grain (up to 1.4-fold). This approach was efficient in improving growth and grain Fe biofortification of wheat in pH affected calcareous soil. | Ramzani et al. (2016) |
| Wheat       | Se          | Grain Se concentration increased up to 26 ng g⁻¹ fresh weight, for each gram of Se ha⁻¹ applied as Na₂SeO₄, while yield and harvest index were not affected by Se fertilization. | Broadley et al. (2010) |
| Wheat       | Se and Zn   | Twenty Brazil wheat accessions (including 15 varieties and 5 cultivars) were used in this study, and Zn and Se concentrations in grains exhibited about twofold and 1.5-fold difference, respectively, between these wheat accessions. The soil Zn application enhanced grain Zn concentration in all accessions up to threefold. The soil Se and Zn application improved Se and Zn concentration in grain and promoted the additional accumulation of Fe. | Souza et al. (2014) |
plants. According to Gonzali et al. (2017), biofortification of food crops can be a cost-effective approach to control deficiency with a bioavailable source. In many plant species, such as potato and lettuce, the agronomic approach is sufficient to increase I content. The most common administration ways are in the soil, as a foliar spray or in hydroponic solutions. The chemical form varies since there are studies with the application of organic and inorganic species. Doses and timing of application must be evaluated for each specie (Gonzali et al. 2017).

In turn, Se biofortification by agronomic strategies such as fertilizer application is an efficient way to produce Se-enriched food products (Wan et al. 2018). However, attention is needed since the levels that characterize deficiency, essentiality, and toxicity of this element are very close (Navarro-Alarcon and Cabrera-Vique 2008). The chemical form of Se influences its bioaccessibility. Fortunately, agricultural methods to improve Se bioaccessibility in food products can be used (Wan et al. 2018). Moreover, the major forms of Se in the diet are highly bioavailable (IOM 2000). According to Wan et al. (2018), agronomic strategies may help supply the daily needs of this element, mainly in Se deficiency regions. Other studies have shown that agronomic Se biofortification of cereals is effective to increase Se intake in animals and humans (Valença et al. 2017). On the other hand, processing methods such as heating and milling may decrease Se content in food due to volatilization and solubilization (Wan et al. 2018; Navarro-Alarcon and Cabrera-Vique 2008).

Zinc biofortification of edible crops has been identified as a strategy to improve the intake of this element. For this purpose, agronomic strategies namely Zn-fertilizers application have been employed and showed to increase Zn content in roots, stems, and leaves without compromising yield. Zinc fertilizers showed promising results when applied either in the soil or in leaves and also in combination with nitrogen fertilizers (White and Broadley 2011). There is evidence that nitrogen availability is a key component of Zn biofortification (Hefferon 2015).

Nevertheless, some studies have shown that other nutrients, such as B, Ca, Cu, Fe, K, Mg, Mn, among others, have also been evaluated for plant biofortification (Aziz et al. 2019; Rizwan et al. 2019; Adu et al. 2018). It is important to mention that in addition to being essential elements for the human organism, they are also very important for the proper development of plants. For this reason, in most cases, the agronomic approaches for crop plant biofortification also improve yield and/or food quality, as described in some studies in Table 7.2.

### 7.3.2 Conventional Breeding and Genetic Approaches

Breeding and genetic engineering are the main tools employed in this type of biofortification (Gonzali et al. 2017). Genetic engineering can employ synthetic genes (Khush et al. 2012). In general, these approaches are more complex and laborious than agronomic ones (Gonzali et al. 2017), but are sometimes needed when conventional methods are insufficient to obtain substantial enhancement of the target element (De Steur et al. 2017). The two methods aim to achieve plant lines
carrying genes that result in the most efficient accumulation of bioavailable minerals. However, plant breeding achieves this by crossing the best performing plants and selecting those with favorable traits over many generations, whereas genetic engineering accesses genes from any source and introduces them directly into the crop (Gómez-Galera et al. 2010).

Plant breeding started more than 10,000 years ago with the selection of seeds to domestication, as occurred to the crops of maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), among others (Dudley 1997). With Mendel’s laws, genetic principles began to be applied to plant breeding, ranging from the introduction, phenotypic selection on natural variants, selection with controlled mating, to marker-assisted selection for desirable genes (Allard 1999). In the beginning, plant breeding was performed unconsciously and deliberately by farmers when they kept some plant of the harvest for planting or sowing their next crop. Besides that, natural selection occurred during the genetic diversity of the crop in new environments, during domestication and subsequent dispersion. Then, the hybridization and genetic-based process were added (Bradshaw 2016). Conventional breeding is possible only between closely related (sexually compatible) individuals, thus relies on natural variation of the target compound within parental lines (De Steur et al. 2015). To increase mineral content by breeding is challenging since numerous genes may be involved in elemental uptake by the roots, translocation throughout the plant, and deposition in edible tissues. Moreover, other factors such as environmental conditions and cultural practices can modify gene expression and alter mineral accumulation by plants (Bouis and Welch 2010).

According to Saltzman et al. (2017) more than 30 countries have officially released biofortified varieties developed using the conventional plant breeding approach, and at least an additional 20 countries have commenced the testing of these varieties and they provide considerable amounts of bioavailable micronutrients, and consumption of these varieties may help to reverse the micronutrient deficiency status among target populations (Saltzman et al. 2017).

Genetic approaches refer to developing crops with improved abilities to acquire and accumulate minerals in edible parts. Modified varieties can also present increased concentrations of “promoter” substances, which stimulate mineral absorption and reduced concentrations of “antinutrients”, substances that negatively interfere with nutrient absorption. However, food’s taste and color may be affected by changes in the concentration of promoters and antinutrients, so these strategies must be cautiously evaluated (White and Broadley 2009). Research and development phases and the regulatory approval process for genetically modified (GM) crops are often time-consuming and expensive. However, after establishment, enhanced crops become sustainable (Khush et al. 2012). Then, in the long term, these strategies can be cost-effective (Gonzali et al. 2017), besides that, they can increase micronutrient concentrations in the desired tissue, such as cereal endosperm, to reduce post milling losses through the outer layers (De Steur et al. 2017). Various genomic approaches, such as quantitative trait loci (QTL) mapping, marker-assisted selection (MAS), marked-assisted recurrent selection (MARS), genome-
wide selection (GS), and next-generation sequencing (NGS) have been widely employed for the biofortification.

Multiples genetic approaches are commonly employed to achieve the best results on mineral biofortification. For instance, Masuda et al. (2012) combined three transgenic approaches to produce Fe-biofortified rice: (1) enhancement of Fe storage in grains via expression of the Fe storage protein ferritin using endosperm-specific promoters; (2) enhancement of Fe translocation through overproduction of the natural metal chelator nicotianamine and (3) enhancement of Fe flux into the endosperm through the expression of the Fe(II)–nicotianamine transporter OsYSL2 expression under the control of an endosperm-specific promoter and sucrose transporter promoter. The authors reported that the Fe concentration of polished seeds increased up to sixfold in greenhouse cultivation and 4.4-fold in paddy field cultivation (Masuda et al. 2012).

Johnson et al. (2011) reported that Fe concentrations were increased, reaching 14 mg kg$^{-1}$, in rice grains by GM. Besides that, Fe was unlikely to be bound by phytic acid and therefore likely to be more bioavailable in human diets (Johnson et al. 2011). Conventional breeding is also an option since there is a natural genetic variation in Zn concentrations of edible crops. Other approaches use genetic engineering to develop modified plants with increased abilities to acquire and accumulate Zn. Still, higher Zn concentrations in edible plant parts can be reached with the development of crops with more tolerance to high Zn levels in tissues. There are already genetically modified plants that have higher concentrations of Zn in the edible parts compared to traditional varieties (White and Broadley 2011). It was noted that plants modified to increase Fe accumulation have also presented increased Zn concentrations. It may indicate a cross-talk between Fe and Zn transport pathways (Hirschi 2009). Connorton and Balk (2019) reviewed several GM crops for iron biofortification, including cassava, maize, wheat, rice, soybean, and sweet potato. The authors also mentioned that several quantitative trait loci and transgenes increase both iron and zinc, due to overlap in transporters and chelators for these two mineral micronutrients (Connorton and Balk 2019).

Considering I biofortification, in some cases, there is a need for genetic engineering strategies to guarantee an effective result. It occurs mainly in cereals because the amount that reaches grains is insufficient to supply human needs. Genetic approaches focusing on reducing I volatilization from leaves or aiming to control the uptake and mobilization of this element through the phloem are promising, but still very scarce. There is a need for reliable protocols for I biofortification of staple crops to enable the dissemination of these practices (Gonzali et al. 2017).

Therefore, the development of genetic biofortification methods must consider the impact that these modifications may have on the accumulation of other elements that are not necessarily the object of the study. Other questions that must be considered are the impact of biofortification on plant metabolism, growth, productivity, environment, and conservation of genetic resources (Garcia-Casal et al. 2017). For instance, enzyme activities may be modified by metal content. Finally, possible alterations of plant stress, interactions with other nutrients, and allergic reactions in humans must be evaluated (Hirschi 2009). The main limitations of genetically
modifying crops included consumers acceptance and to fulfill the regulatory requirements for labeling and approving commercialization of these crops.

### 7.3.3 Plant Growth-Promoting Microorganisms Approaches

Some recent strategies do not fit the previous definitions since they do not include the application of fertilizers during plant growth or even conventional breeding and genetic strategies. The use of plant growth-promoting microorganisms (PGPM), especially the plant growth-promoting rhizobacteria (PGPR), is one of the strategies that has grown significantly in the last years aiming at the crop plant biofortification. The PGPM approaches consist of the application of beneficial microorganisms (bacteria, fungi, among others) in cultivation soil. The soil application of these microorganisms increases mineral bioavailability contributing to crop plant biofortification and improve the soil fertility and crop yield (Khan et al. 2019; Rana et al. 2012). In turn, PGPR consists of a varied group of beneficial bacteria that colonize the rhizosphere and plant roots (Glick 1995). In short, the PGPR is the soil bacteria that stimulate the growth of the host through increasing mobility, uptake, and enrichment of nutrients in the plant (Prasanna et al. 2016). Moreover, they contribute to plant growth development by fixing biological nitrogen, enhancing root function, suppressing disease, among other benefits (Glick 1995; Vessey 2003; Hafeez et al. 2006).

The application of PGPR in agriculture is an attractive way to minimize the use of fertilizers and related agrochemicals (Rana et al. 2012). According to De Santiago et al. (2011), agronomic and genetic approaches have a higher cost than PGPR application, present ethical problems, and are non-environmental friendly. In this way, the use of PGPR agents could be an interesting alternative to agronomic and genetic approaches aiming to promote the crop plant growth as well as enhance the uptake of micronutrients by plants (De Santiago et al. 2011; Mora et al. 2015). Vessey (2003) defined PGPR as biofertilizers, i.e., substances that contain living microorganisms and, once applied to plant or soil, colonizes the rhizosphere or the interior of the plants promoting the increase of supply or availability of primary nutrients to the host plant (Vessey 2003). However, some authors consider that the use of PGPR for crop plant biofortification should be carried out as a possible supplementary measure, along with other approaches (Bouis et al. 2003; Blanchfield 2004). In addition to the use of bacteria, other organisms such as fungi have also been used for this purpose (Durán et al. 2013). In Table 7.3 are presented some studies demonstrating the application of microorganism strains to the soil for crop plant biofortification.

According to the studies described in Table 7.3, it is possible to verify that the application of microorganism strains to the cultivation soils, especially for cereals and legumes, is a promising approach for mineral biofortification. Although the combination of agronomic and PGPR approaches can be an advantageous alternative for crop plant biofortification, in some cases only the application of microorganism strains to the soil may promote the same benefits. The application of strains of
Table 7.3  Studies demonstrating the use of plant growth-promoting microorganisms for mineral biofortification of crop plants

| Crop plants | Elements          | Results                                                                 | Reference                      |
|-------------|-------------------|------------------------------------------------------------------------|--------------------------------|
| Chickpea    | Ca, Cu, Fe, Mg, Mn, and Zn | The potential of plant growth-promoting actinobacteria in increasing seed mineral density of chickpea under field conditions was evaluated. Nineteen isolates of actinobacteria were tested, and for all them, mineral concentration was higher than those observed for uninoculated control treatments. Concentration of Ca, Cu, Fe, Mg, Mn, and Zn were up to 26%, 54%, 38%, 21%, 35%, and 30%, respectively. | Sathya et al. (2016) |
| Chickpea    | Fe                | Five bacterial isolates were evaluated for improving plant growth and bioavailable Fe concentration in chickpea. Application of the PGPR significantly enhanced the plant height, root length, root fresh and dry weights, shoot fresh and dry weights. Besides, the inoculated plants presented Fe concentration higher than those obtained for uninoculated control plants. Application of PGPR along with FeSO₄ (as fertilizer) showed 81% and 75% increase in grain and shoot Fe concentration, respectively, when compared to control (uninoculated plants) | Khalid et al. (2015) |
| Chickpea and pigeonpea | Ca, Cu, Fe, Mg, Mn, and Zn | Seven strains of bacteria were evaluated for improving plant growth and biofortification in chickpea and pigeonpea under field conditions. Evaluated bacteria significantly enhanced the shoot height and root length of both chickpea and pigeonpea over the uninoculated control. Besides, mineral concentration in the harvested grains from the inoculated plants were higher than those observed for uninoculated control treatments—was up to 22% and 11% for Ca, 19% and 8% for Cu, 18% and 12% for Fe, 2% and 39% for Mn, and 23% and 5% for Zn, in chickpea and pigeonpea, respectively. | Gopalakrishnan et al. (2016) |
| Mung bean   | Fe                | Two strains of bacteria were evaluated, and results showed that both have a high chelating potential for iron. Also, pot study results revealed a significantly increased in vegetative parameters, Fe concentration (up to 3.4-fold), protein (up to 2.5-fold) and carbohydrates (up to 1.5-fold) in inoculated plants, demonstrating the potential of this approach for plant growth and Fe biofortification in mung bean. | Patel et al. (2018) |

(continued)
Table 7.3 (continued)

| Crop plants | Elements | Results                                                                                                                                                                                                 | Reference                     |
|-------------|----------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------|
| Rice        | Fe       | Three plant growth-promoting rhizobacterial strains isolated from rhizospheric soils were applied to field-grown rice plants for improving Fe concentration of grains. The results showed that grain Fe concentration almost doubled, and the Fe translocation efficiency from roots to shoots to grains significantly enhanced. The authors emphasize that the application of PGPR strains is a promising strategy to combat the problem related to Fe deficiency in rice and consecutively in human masses. | Sharma et al. (2013)           |
| Soybean and wheat | Zn   | Three strains of bacteria were assessed for improving crop growth, and the mobilization and biofortification of Zn. Results demonstrated that Zn concentration in shoots and roots had an increase of up to 23% and 29% for soybean and 68% and 49% for wheat, respectively. Also, the evaluated strategy increased the yield of both crops and, therefore, can be used for biofertilization and biofortification. | Ramesh et al. (2014)           |
| Wheat       | Cu, Fe, Mn, and Zn | One bacterial and three cyanobacterial strains were evaluated in a field experiment. Results demonstrated that bacterial strain improves the nutritional quality of wheat grains. The concentration of Cu, Fe, and Mn increased by up to 150%, 105%, and 37%, respectively. The concentration of Zn was similar to those obtained in control (only NPK fertilizer application) for all evaluated strains. | Rana et al. (2012)             |
| Wheat       | Fe and Se | The effect of bacterial inoculation and selenate fertilization on Se uptake and plant growth was assessed. Inoculation with YAM2 (a bacterium with 99% similarity to Bacillus pichinotyi), both in the presence and absence of selenate, showed significantly higher dry weight, shoot length, and spike length compared to uninoculated plants. Se concentration in inoculated plants was considerably higher in wheat kernels (167%) and stems (252%) when compared to uninoculated plants. Similar behavior was observed in Fe concentration for inoculated plants that have an increase in kernels (70%) and stems (147%). | Yasin et al. (2015)            |
| Wheat       | Fe and Zn | Four bacterial isolates were in vitro studied and, further, in field experiments on two varieties of Triticum aestivum. The strains                                                                 | Shaikh and Saraf (2017)        |

(continued)
bacteria in soil, for example, was effective in increasing the Ca, Cu, Fe, Mg, Mn, and Zn concentration of chickpeas and wheat without the need to add fertilizers avoiding problems related to environmental pollution (Rana et al. 2012). Moreover, the use of fungi strains has also been promising. Durán et al. (2013), for example, observed an increase of 24% in Se concentration in wheat co-inoculate with a mixture of rhizobacteria and arbuscular mycorrhizal fungi. It is important to note that, in both cases, the application of microorganism strains to soil would not have the same success if were performed in mineral-deficient soils. Even so, in these cases, the use of PGPM is an environmentally friendly and low-cost alternative that, associated with agronomic approaches, may provide savings regarding the use of fertilizers.

### 7.4 Is Biofortification a Solution to Tackle Elemental Deficiency?

It is known that hidden hunger or micronutrient deficiency is a worldwide concern, leading to about two billion people who do not have access to supplements or a diversified diet to consequences such as anemia and even death (HarvestPlus 2020). It is also known that biofortification strategies are sustainable and effective tools to improve the nutritional status of staple crops (Díaz-Gómez et al. 2017). In the last
years, it was possible to observe significant progress in research and development of biofortified foods, with a variety of new strategies emerging for several nutrients/crops (Hefferon 2015). However, biofortification efficiency to tackle elemental deficiency in humans is not yet a fully clarified subject, generating controversies among researches. There is a lack of nutritional assessment regarding biofortified foods and their impact on global human health.

Valença et al. (2017) highlighted that, despite the potential of biofortification to increase nutritional content and yield of food crops, more evidence is necessary to prove its influence in human health and its efficacy to alleviate micronutrient deficiencies. Another point is that biofortification strategies must be adapted for different staple crops that are commonly harvested in each region. Moreover, the success of biofortification is related to the correct choice of food preparation and cooking methods that can impact on nutrient bioavailability (Díaz-Gómez et al. 2017). Another challenge that must be overcome is the public perception of biofortification, which may influence the regulation and implementation of genetically modified crops (Hefferon 2015). Thus, before these techniques are widely applied, its influence on nutrient bioavailability must be confirmed (Díaz-Gómez et al. 2017). Beyond that, systematic research and comprehensive feeding trials are needed to clarify the benefits that they can have on human health in the long term. Finally, the impacts of these foods must be assessed in the fields of nutrition, health, environment, and agriculture (Hirschi 2009).

On the other hand, a review conducted by White and Broadley (2009) concluded that biofortification of crop plants has a great potential to improve the nutritional status of humans, without compromising crop yield. Khush et al. (2012) and Díaz-Gómez et al. (2017) agreed that biofortification is a promising tool to alleviate malnutrition in vulnerable populations. Biofortification is one of the tools to combat hidden hunger by increasing the micronutrient content of staple foods (HarvestPlus 2020). Both Food and Agriculture Organization of the United Nations (FAO) and HarvestPlus, which is part of the CGIAR Research Program on Agriculture for Nutrition and Health (A4NH) and is led by the International Food Policy Research Institute (IFPRI), have been working together in the development, production, and implementation of biofortified staple crops aiming to improve nutrition and health of vulnerable populations. Iron, zinc, and vitamin A are the main focus of these programs where biofortification is carried out through conventional crop breeding. In general, the target foods are stapling crops such as rice, maize, wheat, cassava, beans, and sweet potato. The adoption and expansion of biofortification programs are highly encouraged and supported by the aforementioned agencies (HarvestPlus 2020).

For the success of a biofortified crop, tests must be carried out to scientifically prove that it will indeed contribute to the increase in micronutrient intake. Only after this stage, the biofortified crop can be disseminated and consumed as a safe and effective nutrient source. One of the advantages of these crops is that they can be continuously improved after implementation, since varieties with superior qualities, such as the higher concentration of micronutrients, can be always selected (HarvestPlus 2020).
According to HarvestPlus (2020), biofortified crops of 200 varieties are already officially present in 30 countries (HarvestPlus 2020). For example, in 2019 there were 39 varieties of iron-biofortified beans released in Africa and 21 in Latin America and the Caribbean. These beans, when consumed as a staple, would supply 80% of the estimated average requirement (EAR) for Fe. Also, a total of ten varieties of pearl millet and eight of cowpea biofortified with Fe were released, supplying 80% and 25% of the iron EAR, respectively. In 2019, Zn biofortified crops (11 varieties of wheat, 10 of rice, and 7 of maize) were legalized, providing respectively 50%, 40%, and 70% of the EAR of Zn. The overall climate-adaptiveness and higher yields of biofortified crops contributed to its acceptance by farmers (HarvestPlus 2018). By 2018, biofortified crops such as iron beans and zinc rice were grown by about 7.6 million farmers (HarvestPlus 2018, 2019). Consumers usually have a good acceptance of biofortified crops, enjoying its taste, appearance, odor, and texture (HarvestPlus 2020). A total of 38 million people were growing and consuming biofortified crops in 2018 (HarvestPlus 2018).

Many studies have shown the nutritional and health benefits of biofortified crops, mainly to people who consume then as staple foods. These studies found that nutrients in biofortified crops are as bioavailable as those of traditional varieties. The consumption of these crops can improve micronutrient status, cognitive function, and reduce morbidity, as well as supply 80% of the daily average requirement of Fe and 70% of Zn (HarvestPlus 2020).

A study conducted with Rwandan women suggested that the consumption of Fe-biofortified beans contributed to the improvement of iron status and to prevent and reverse iron deficiency among those women (Haas et al. 2016). Scott et al. (2018) performed an intervention study in 140 Indian boys and girls, aged 12–16 years old, concluding that the consumption of iron-biofortified pearl millet improved Fe status as well as some measures of cognitive performance (memory and attention) (Scott et al. 2018).

Brnić et al. (2015) have compared the zinc absorption from a rice variety fortified with Zn and the same rice variety biofortified with zinc. The results showed that rice biofortification was as good as the postharvest fortification to combat zinc deficiency and biofortified rice presented more bioavailable zinc than conventional rice (Brnić et al. 2015). A study conducted with 6005 participants suggested that the consumption of zinc-biofortified wheat reduces maternal and child morbidity (Sazawal et al. 2018).

In conclusion, scientific research, development, and application studies have suggested that biofortification can contribute to more people having access to a healthy and diverse diet by making staple crops more nutritious. It contributes to the improvement of the nutritional status of vulnerable populations and helps fight hidden hunger. Moreover, there is evidence that farmers and consumers have accepted biofortified foods well (HarvestPlus 2020). Then, biofortification together with other approaches namely supplementation, dietary diversity, and food fortification are complementary strategies to tackle elemental deficiency.
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