

Chapter 9



Selenium biofortification for human and animal nutrition

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9.1 INTRODUCTION

Selenium (Se) is an essential trace element, playing a crucial role in the functioning of enzymes in humans and animals and protecting cells from damage by free radicals (Hatfield *et al.*, 2014). Selenoproteins, that is, proteins containing selenium, are best known as antioxidants and catalysts for the production of active thyroid hormone (Rayman, 2012). Although the essential role of Se for the growth and survival of plants has not been confirmed yet, it is a beneficial element for plants, which can enhance resistance to stress (see Chapter 8).

Despite the importance of this trace element, intake of Se by animals and humans in a wide range of countries, including several countries in Western Europe and East and Central Africa, is still low, resulting in Se deficiency and causing negative health effects, including increased risk of mortality, poor immune function, and cognitive decline (Broadley *et al.*, 2006; Rayman, 2012; Roekens *et al.*, 1986). An estimated one billion people around the world are affected by selenium deficiency, because of low Se intake (Poblaciones & Rengel, 2017; Rayman, 2004). The recommended daily Se intake in an adult human diet is 0.04–0.4 mg per person per day (Food and Agriculture Organization of the United Nations/World Health Organization [FAO/WHO], 2001). Besides, farm animals (Dermauw *et al.*, 2013) and pets (van Zelst *et al.*, 2016) can be affected by Se deficiencies, leading to economic losses. Therefore, the Se content in the human

and animal diet is a topic of interest to public health systems around the world (Lavu *et al.*, 2012).

Biofortification, that is, the dietary supply of Se through its enrichment in food and feed crops, is being explored as a possible solution for Se deficiency (Lavu *et al.*, 2013; Li *et al.*, 2010; Thavarajah *et al.*, 2008). This chapter gives insights into factors affecting Se toxicity and deficiency for humans and animals, and meanwhile summarizes the different phytotechnologies used for Se biofortification, including conventional plant breeding and genetic engineering, and soil and foliar application of Se-based fertilizers (agronomic biofortification), with specific attention to the use of Se-enriched organic materials and nano-sized selenium (SeNPs), and the addition of beneficial microorganisms into soil for enhancement of Se accumulation in the crops. The factors influencing Se biofortification strategies are also discussed.

9.2 SELENIUM TOXICITY AND DEFICIENCY FOR HUMANS AND ANIMALS

Selenium exists in inorganic forms as selenate (SeO_4^{2-}), selenite (SeO_3^{2-}), selenide (Se^{2-}), elemental Se (Se^0), and in organic forms such as selenocysteine (SeCys) and selenomethionine (SeMet). Due to this diversity in form of occurrence, Se is found in all natural materials on Earth: soil, rocks, waters, air, plants and animals (Fordyce, 2007). For a long time, Se has been identified as a dangerous substance because of its toxicity (Fordyce, 2007). More recently, it has also been recognized as an essential trace element due to its crucial role in the functioning of enzymes of humans and animals (Fordyce, 2013; Rayman, 2000). The range between beneficial and harmful Se concentrations is relatively narrow for animals and humans (Li *et al.*, 2015a). Thus, both toxic and deficient incidences of Se dietary uptake have been reported over the world (Li *et al.*, 2015a).

9.2.1 Se toxicity

Se intoxication events for animals and humans, such as selenosis in America, Canada, China, and Mexico, have occurred occasionally where Se has entered the food chain in excessive amounts (Li *et al.*, 2015a). These events were caused by excessive Se in soil and water. For instance, the Se toxicity for humans and animals discovered in the Enshi District, Hubei Province, and in Ziyang County, Shanxi Province in China was related to the extremely high Se concentrations in the local food and environment (Fordyce *et al.*, 2000). For humans, Se toxicity (selenosis) can result in garlic breath, hair and nail loss, nervous system disorder, poor dental health, and paralysis (Rayman, 2012). For animals, excess Se can cause alkali disease and blind staggers for grazing animals, and hoof loss in hooved animals (Fordyce, 2007; Tan *et al.*, 2002). Alkali disease is characterized by dullness, lack of vitality, emaciation, rough coat, sloughing of the hooves,

erosion of the joints and bones, anemia, lameness, liver cirrhosis, and reduced reproductive performance (Fordyce, 2007). Blind staggers results in impaired vision and blindness, anorexia, weakened legs, paralyzed tongue, labored respiration, abdominal pain, emaciation, and death (Fordyce, 2007). Hair loss and other abnormalities in farm animals have also been observed in areas of Columbia, as a result of Se toxicity (Johnson *et al.*, 2009).

9.2.2 Se deficiency

On the contrary, Se deficiency is also frequently observed worldwide and is even more widespread than Se toxicity. It is estimated that 0.5–1 billion people are directly affected by Se deficiency on a global scale due to low dietary Se intake (Haug *et al.*, 2007; Stonehouse *et al.*, 2020). It has been demonstrated that Se deficiency can cause Keshan disease and Kashin-Beck disease (endemic disease) with low Se supplies in the food system, that is, weakening of the heart and also atrophy and necrosis of cartilage tissue in the joints, which has been reported in the middle of China (Stone, 2009), Saudi Arabia, the Czech Republic, Burundi, New Guinea, Nepal, Croatia, and Egypt (Wu *et al.*, 2015). Low Se status has also been associated with a significantly increased risk of cancer incidence and mortality, cardiovascular risk, poor immune function, male infertility and lower reproduction (Fordyce, 2007; Haug *et al.*, 2007). In addition, Se deficiency may also be a factor in some other diseases. For instance, studies have found that the prevalence of iodine deficiency diseases was greater among populations with lower Se status than among those with higher Se status in Africa (Combs, 2001). This is probably attributed to the fact that Se is essential for the metabolic production of thyroid hormone.

Se deficiency adversely affects livestock health around the world, including south and north America, Africa, Australia, UK and New Zealand (Reilly, 1996). Selenium deficiency causes reproductive and immune response impairment of animals, growth depression (ill-thrift), and white-muscle disease, a myopathy of heart and skeletal muscle principally affecting cattle, sheep, poultry and horses (Rayman, 2000).

Se deficiency in humans and animals is attributed to a low Se daily dietary intake, varying considerably between regions. As mentioned previously, Se deficiency has been identified in parts of the world which have a notably low content of Se in soil or water, as Se enters the food chain from the environment through crop and plant uptake, mainly from local water or soil (Haug *et al.*, 2007). Therefore, the Se concentration in foods is determined by geological and geographical factors. Globally, the total Se concentration in soils ranges from 0.01 to 2.0 mg/kg, with a mean of 0.4 mg/kg (He *et al.*, 2010; Rayman, 2008). Some parts of the world have relatively low Se contents in their soils such as Denmark, Finland, New Zealand, eastern and central Siberia and a long belt extending from northeast to southwest China including parts of Heilongjiang, Jilin, Liaoning, Hebei,

Shanxi, Shaanxi, Sichuan and Zhejiang provinces and Inner Mongolia. Therefore, these regions are characteristic for low amounts of Se in their food chains (Combs, 2001).

9.2.3 Se in nutrition

In humans, chronic Se toxicity is observed above levels of 400 µg/day and Se deficiency occurs when the dietary intake of Se is below 40 µg/day (Gupta & Gupta, 2017; Winkel *et al.*, 2012). More specifically, the tolerable upper intake levels are 90 µg/day for children of 1–3 years, 150 µg/day for children of 4–8 years, 280 µg/day for children of 9–13 years, and 400 µg/day for children >14 years and adults (Ngigi, 2019; Sciences, 2000). For livestock, the toxic Se concentration in animal feed is approximately 2–5 mg/kg dry forage, while the minimal requirement of Se is defined as 0.05–0.10 mg/kg (Gupta & Gupta, 2017). The National Research Council (NRC, 2005) has published the following maximum tolerable levels (MTL) for animals: 5 mg Se/kg feed dry matter (DM) for cattle and sheep, 4 mg Se/kg feed DM for pigs, and 3 mg Se/kg feed DM for poultry. The MTL for horses and fish were derived from interspecies extrapolation and amount to 5 and 2 mg Se/kg DM feed, respectively (NRC, 2005).

Table 9.1 summarizes the recommended daily Se intake and Table 9.2 overviews the status of daily Se intake in some countries. The two tables show that the

Table 9.1 Recommended daily Se intake for adults (µg/d).

Countries	Males	Females
Australia (1990)	85	70
Belgium (2000)	70	70
France (2001)	60	50
FAO/WHO (2001)	40	40
Germany, Austria, Switzerland (2013)	30–70	30–70
Italy (1996)	55	55
Japan (1999)	55–60	45
Netherlands (2000)	50–150	50–150
Nordic countries (2014)	60	50
Ireland (1999)	55	55
Scientific Committee Food (2003)	55	55
USA and Canada (2000)	55	55
United Kingdom (1991)	75	60

Adapted from: EC Scientific Committee on Food (2003); Thomson (2004); Rayman (2004) and European Food Safety Authority (EFSA, 2014).

Table 9.2 Estimated selenium intake status of adults in several countries ($\mu\text{g}/\text{person}$ per d).

Countries	SE Intake
Australia	57–87
Austria	48
Belgium	28*–61
Canada	98–224
China	
Keshan disease area (e.g., a wide belt from northeast China to southwest China)	7–11*
Moderate Se area (e.g., Guangzhou)	40–120
Selenosis area (e.g., Hubei and Shaanxi provinces)	750–4990
Czech Republic	10–25*
Denmark	38*–47
Finland	
Before 1984	25*
After 1984 (Se biofortification)	67–110
France	29*–43
Germany	35*
Ireland	44–59
Italy	35*–42
Japan	104–199
Latvia	50
New Zealand	55–80
Serbia	30*
Slovakia	27*–43
Sweden	38*
Switzerland	70
UK	29*–39*
USA	60–220

Table adapted from: [Combs \(2001\)](#); [Rayman \(2004\)](#) and [EFSA \(2014\)](#).

* indicates that this level does not meet the WHO recommended requirement ([FAO/WHO, 2001](#)).

recommended daily Se intake in some countries has not yet been achieved, such as in some European countries and parts of China. This demonstrates that the food systems of these countries do not provide sufficient Se for consumption. It may thus be assumed that many individuals have a potential risk of Se deficiency,

which can increase their risks to various diseases, including those of the heart and lungs, as well as cancer, and make them more vulnerable to infectious diseases due to poor functioning of their immune system. There is a clear need to enhance Se in food systems of these countries to remediate Se deficiency.

9.3 SELENIUM BIOFORTIFICATION STRATEGIES FOR ADDRESSING Se DEFICIENCY

Addressing micronutrient deficiencies to reduce health-related issues can be achieved through various types of interventions, such as through food supplements, dietary diversification, biofortification, or increasing the digestibility of trace elements in foods and products (Lavu *et al.*, 2012; Li *et al.*, 2020). For instance, sodium selenite has been supplemented in feeds in some areas with selenium deficiency in livestock in order to achieve optimal Se intake (EFSA, 2016). Biofortification is one of the most promising, widespread and accepted strategies, aimed at improving the lack of Se in a diet through enrichment of food and feed crops, in particular the edible parts of plants using different phytotechnologies (Snchez *et al.*, 2017). The different strategies are summarized in Figure 9.1.

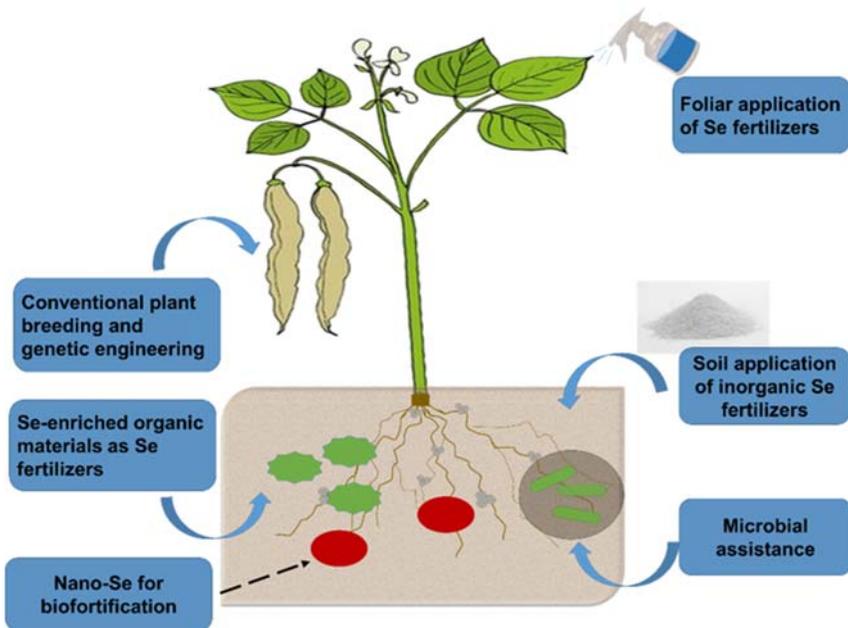


Figure 9.1 Overview of Se biofortification strategies.

9.3.1 Conventional plant breeding and genetic engineering

Breeding of crops aims to screen plant varieties with specific traits, such as the ability for elevated Se uptake or accumulation, to transform Se from inorganic to organic species or to translocate Se quickly from the roots to edible parts. This approach has been explored as a practice for the enhancement of the Se content in edible plants, because there is a huge interspecies and intraspecies genetic variation in plants (Schiavon *et al.*, 2020). Previous studies have demonstrated genetic variation in grain Se concentration of cereal crops, such as wheat (Sharma *et al.*, 2016; Wang *et al.*, 2021; White, 2016), rice (White, 2016; Zhang *et al.*, 2006), oat and barley (White, 2016). Moreover, significant genetic variation effects on seed Se concentration of leguminous crops and edible parts of vegetables have also been observed. Leguminous crops include common bean, mung bean, field pea, lentil and chickpea, and vegetable species include onion, broccoli, Brassicaceae *spp.*, lettuce, tomato, pepper, Chinese cabbage, Indian mustard, pepper, cauliflower and potato (see the review of White (2016) for specific references).

Besides conventional breeding, genetic engineering, as an emerging cutting-edge technology, has shown promise to improve the Se biofortification efficiency (Schiavon *et al.*, 2020). It is aimed at enhancing Se accumulation, preferentially as beneficial selenoamino acids, in the edible part of plants via transgenic methods (White, 2016). So far, genetic engineering for improvement of biofortification has focused on genetic manipulation for (1) reduction of selenate in plants through overexpression of sulfate transporters or adenosine triphosphate (ATP) sulfurylase (APS1 or APS2), which can catalyze the rate-limiting steps of Se assimilation in plants resulting in higher Se accumulation, (2) conversion of Se-cystine (SeCys) to Se-methionine (SeMet) and dimethyl selenide (DMS₂) by Cystathionine- γ -synthase (CSeGS) enzyme, leading to more Se volatilization, and (3) avoidance of SeCys misincorporation into proteins by mouse selenocysteine lyase or SeCys methyltransferase (SMT) enzyme (Sarwar *et al.*, 2020; Schiavon *et al.*, 2020; Zhu *et al.*, 2009).

Transgenic lines of Indian mustard (*Brassica juncea* L. Czern.) overexpressing genes encoding the enzymes APS, γ -glutamyl-cysteine synthetase (ECS) and glutathione synthetase (GS) were tested under field conditions. The APS, ECS, and GS transgenic plants accumulated 4.3, 2.8, and 2.3 fold more Se in their leaves than the wild type, respectively (Bañuelos *et al.*, 2005). Furthermore, the overexpression of the SMT gene (identified from the Se accumulator *Brassica juncea* L.) in tobacco plants could substantially enhance the tolerance to selenite stress, as shown by the significantly higher fresh weight, plant height, and chlorophyll content than control plants (Chen *et al.*, 2019). More importantly, transgenic plants accumulated a high level of Se and the selenoamino acid Se-methyl-selenocysteine (MeSeCys) (Chen *et al.*, 2019). Genetic engineering can thus improve Se accumulation and give a higher yield with better nutritional

quality for biofortification purposes. However, the limitation of plant breeding and genetic engineering is that these have to be applied combined with agronomic Se biofortification, particularly for plants grown in Se deficient regions.

9.3.2 Agronomic biofortification

9.3.2.1 Soil inorganic Se fertilizer application

The agronomic approach of applying a fertilizer on the soil can improve the nutritional quality of the plant without genetic modifications (Storksdieck & Hurrell, 2009). It has been developed as a food-based method to help decrease widespread deficiencies of Se. Selenite and selenate-based fertilizers are typically applied (as granular/blended forms or liquid drenches) into soil to improve their total and bioavailable Se, subsequently resulting in a higher Se concentration in the crop. Although Se is not an essential trace element for plants, it presents chemical similarity to S, and both elements have the same carrier membranes and biochemical pathways for assimilation in the plant (see Chapter 8). Soil application of Se fertilizers can therefore ensure its sufficient concentration in the edible parts of plants (Prado *et al.*, 2017; Sarwar *et al.*, 2020).

Se biofortification of food crops is already successfully practiced in some countries (Se-deficient regions) to increase the Se concentration in staple grains, and subsequent dietary Se intake, by adding inorganic Se fertilizer to soils (Bañuelos *et al.*, 2016; Broadley *et al.*, 2006). For instance, in Finland, a three-fold increase of mean Se intake was observed after agronomic Se biofortification in the form of selenate within 2 years, and the concomitant human serum Se concentration was increased by 70% (Aro *et al.*, 1995). Several plants have been successfully biofortified with Se, such as wheat (Ali *et al.*, 2017; Mao *et al.*, 2014), maize (D'Amato *et al.*, 2019; Mao *et al.*, 2014), rice (Gong *et al.*, 2018; Pandey & Gupta, 2015), soybean (Mao *et al.*, 2014), cabbage (Seo *et al.*, 2008), canola (Mao *et al.*, 2014), potato (de Oliveira *et al.*, 2019), lettuce (Munier-Lamy *et al.*, 2007), pak choi (Li *et al.*, 2015a), and tomato (Carvalho *et al.*, 2003). It should be noted that selenate is superior over selenite for soil application, as selenate is highly soluble and bioavailable in soil, while selenite is less mobile and easily absorbed on oxide surfaces, resulting in less bioavailable Se for plants (Schiavon *et al.*, 2020).

9.3.2.2 Foliar Se fertilizer application

Being an alternative to soil application, foliar spraying of Se fertilizers can efficiently and economically improve Se concentrations in crops. With this method, Se-based fertilizers are homogeneously sprayed on plants. Studies showed that the efficiency of foliar Se application is on average eight times higher than soil application, suggesting that foliar application is preferred over soil application (Ros *et al.*, 2016). This is attributed to (1) liquid Se-containing fertilizer being directly applied onto plants via spraying, which avoids the

retention of Se by the soil (e.g., binding by soil organic matter), thus improving Se utilization by plants and avoiding Se losses; and (2) the translocation of Se from root to shoot or edible parts of plants not being required through the foliar application, resulting in fast assimilation of Se in plant tissues (Schiavon *et al.*, 2020). Foliar application of Se-based fertilizers has successfully enhanced the Se concentration in many plants, including cereal crops: rice (Farooq *et al.*, 2019), wheat (Wang *et al.*, 2020b), maize (Ngigi, 2019) and beans (Ngigi *et al.*, 2019) as well as vegetable crops: tomato (Zhu *et al.*, 2016), potato (Zhang *et al.*, 2019), cabbage, radish, onion and garlic (Slekovec & Goessler, 2015).

Some practical aspects should be carefully considered when implementing foliar application, such as the applied Se dose, the timing of the foliar Se application, and the plant type. For instance, phytotoxicity could be caused by an unsuitable Se concentration sprayed directly on plant leaves. The plants that received Se-based fertilizer should have sufficient leaf area for maintaining and absorbing Se during the biofortification process, and suitable weather during application should be considered in order to avoid Se losses on rainy and windy days. Besides, the application timing is another item that should be addressed, as an application at different growth stages can result in different Se accumulation by the plants. Wang *et al.* (2020b) demonstrated that foliar application of selenate or selenite at the pre-filling stage was superior in improving the Se concentration of wheat grains than that at the pre-flowering stage. The foliar application of selenite during the potato tuber bulking stage resulted in the greatest Se accumulation in the tubers, compared to the application during the tuber initiation and maturation stages (Zhang *et al.*, 2019).

9.3.2.3 Novel Se fertilizers

9.3.2.3.1 Se-enriched organic materials as Se fertilizers

Biomaterials, such as plant residues, sludge and manures, that come from seleniferous areas potentially contain high levels of Se. These micronutrient-enriched materials may serve as potential micronutrient sources and can thus be utilized for Se biofortification of agricultural crops. If Se-enriched organic biomaterials are used to amend agricultural soils, their decomposition will gradually lead to micronutrient release into the soil solutions, which will become bioavailable for uptake by the crop (Bañuelos *et al.*, 2015). Biofortification using these Se-enriched biomaterials can thus be achieved, particularly of crops growing on micronutrient-deficient soils (Bañuelos *et al.*, 2016; Li *et al.*, 2017).

Some studies have investigated the possibility of using Se-enriched biomaterials as fertilizer to improve the Se concentration in crops for biofortification purposes. The accumulation of Se in canola, grown on soil amended with 1.5 mg/kg seleniferous *Astragalus praelongus* E. and *Medicago saliva* L. tissues, was increased as the application dose of these materials increased (Ajwa *et al.*, 1998). Se-enriched wheat and Raya plant straw were used to biofortify sorghum, maize

and berseem (Dhillon *et al.*, 2007), and results showed that the Se concentrations in the plants were consistent with the trend of soluble Se in the soil. Similarly, Se-enriched duckweed, Se-enriched anaerobic granular sludge (Li *et al.*, 2021a) and Se-enriched microalgae (Li *et al.*, 2021b) generated in wastewater treatment systems have been evaluated as potential Se fertilizers for improvement of the Se concentration in green beans (*Phaseolus vulgaris*). These biomaterials produced during wastewater treatment released Se, which was efficiently taken up by the beans without negatively affecting their yield. Application of 0.45 g Se-enriched microalgae biomass into 0.5 kg sandy soil even stimulated the beans growth, resulting in a 43% higher yield (Li *et al.*, 2021b). Se-enriched sludge was found to be the preferred slow-release Se biofertilizer for Se-deficient areas, in comparison to Se-enriched duckweed because Se contained in the Se-enriched sludge was released slowly and was more bioavailable for plant uptake than Se contained in the duckweed (Li *et al.*, 2021a).

The supplementation of soils with Se-enriched organic materials as biofertilizer does not only improve the Se concentration in the plants, but also results in value-added plant-based products, as plants can transform the Se taken up during growth into valuable organic Se species (e.g., SeMet, SeCys and MeSeCys), which have important assets in the nutrition of animals and humans. Bañuelos *et al.* (2015) reported that the Se concentration in the edible parts of broccoli and carrots was directly correlated with the amount of Se-enriched *Stanleya pinnata* applied into coarse-loamy soil ($R^2 = 0.94$) and that MeSeCys was the main accumulating Se species. Likewise, the application of Se-enriched duckweed and sludge into loamy and sandy soil as Se biofertilizer significantly improved the proportion of health-beneficial selenoamino acids (e.g., Se-methionine, 76–89%) in the seeds of beans (*Phaseolus vulgaris*) (Li *et al.*, 2021a).

One of the main advantages of micronutrient-enriched organic materials is that they provide a long-lasting micronutrient source, slowly releasing the micronutrients along with the decomposition of the organic materials in the soil (Ajwa *et al.*, 1998). However, the disadvantage is that the application of these materials can introduce additional organic matter into the soil, which can lead to the immobilization of other nutrients in the soil, eventually decreasing the bioavailability for the plant (Stavridou *et al.*, 2011).

It should be noted that Se biofortification via the application of Se-enriched organic materials may not be feasible in all Se-deficient areas. For instance, the Se-deficient region in northeast China, characterized by a high content of organic matter in soil, is not suitable for supplementation with micronutrient-enriched organic materials, as the presence of too much organic matter in the soil will increase the retention of the released Se, reducing the bioavailability of Se in the soil. In contrast, some regional soils with strong leaching potential (i.e., high precipitation (rainfall) and humid climates) and low Se content can benefit from the addition of micronutrient-enriched organic materials since the added organic matter can act as a micronutrient reservoir to

avoid leaching of nutrients and their mobilization to the deeper soil layers (Wang & Gao, 2001).

9.3.2.3.2 Nano-Se for biofortification

In recent years, the application of Se nanoparticles (SeNPs) has been proposed for Se biofortification, as SeNPs can slowly release Se for plant uptake, thus minimizing Se losses in comparison with the fast leaching of inorganic Se (El-Ramady *et al.*, 2020). SeNPs can be synthesized from oxidized Se forms (i.e., selenite and selenate) via chemical or biological reduction using chemical reducing agents (El-Ramady *et al.*, 2020) or bacteria (Staicu *et al.*, 2015), fungi (Mosallam *et al.*, 2018) and plants (Ikram *et al.*, 2021; Schiavon *et al.*, 2020). The effects of SeNPs on plants highly rely on the particle size and synthesis method (chemosynthesized or biosynthesized) of the SeNPs (Hu *et al.*, 2018). Previous studies have identified the potential of SeNPs to promote plant growth, increase Se uptake and improve plant quality (Domokos-Szabolcsy *et al.*, 2012; Hussein *et al.*, 2019). The beneficial effects of SeNPs have been shown for several plants, including tomato (Hernandez-Hernandez *et al.*, 2019; Morales-Espinoza *et al.*, 2019), pomegranate (Zahedi *et al.*, 2019), wheat (Hu *et al.*, 2018), rice (Wang *et al.*, 2020a), garlic (Li *et al.*, 2020) and tobacco (Domokos-Szabolcsy *et al.*, 2012). Besides, SeNPs have lower toxicity for plants compared to selenite and selenate (El-Ramady *et al.*, 2020; Li *et al.*, 2020). SeNPs taken up by plants were quickly oxidized to selenite and transformed to organic Se species (e.g., SeCys, SeMet and MeSeCys) in the plant root (Hu *et al.*, 2018; Wang *et al.*, 2020a). However, further investigations are still required to understand the phytotoxicity of SeNPs on various plants and the potential risks to the environment of using SeNPs.

9.3.2.4 Microbial assistance of biofortification

A novel approach in biofortification studies is to make use of plant-microbe interactions for improvement of Se uptake by the plant. Plant growth promoting rhizobacteria (PGPR) not only promote plant growth via different plant-driven mechanisms, for example, the production of phytohormones, nitrogen fixation and stress mitigation, but also affect the Se mobility, speciation and bioavailability in soils (Sarwar *et al.*, 2020; Yasin *et al.*, 2015a).

Early studies have reported that the addition of beneficial microorganisms to soil or inoculation of plants with microbes could enhance Se accumulation in crops. For instance, Yasin *et al.* (2015a) demonstrated that inoculation of wheat plants with both selenium-tolerant bacterial strains *Bacillus cereus*-YAP6 and *Bacillus licheniformis*-YAP7 not only significantly enhanced wheat growth, but also increased the uptake of Se and other nutrients, for example, S, Ca and Fe. Yasin *et al.* (2015b) further reported that the inoculation of the bacterial consortium G1 stimulated the growth of the Se accumulator Indian mustard (*Brassica juncea*) grown on seleniferous soil for Se-enriched plant material production, which

resulted in a higher Se accumulation. Similarly, inoculation of the arbuscular mycorrhizal fungi increased the Se content in the shallot bulb (*Allium cepa* L. *Aggregatum* group) by 530% (Golubkina *et al.*, 2019).

9.4 FACTORS AFFECTING Se BIOFORTIFICATION EFFICIENCY

Since low concentrations of plant Se can decrease the dietary intake of Se, it is vital to increase Se uptake by plants and to produce plants with higher Se concentrations and bioavailability in their edible tissues (Bañuelos *et al.*, 2017). This is the key issue for effectively developing a biofortification strategy. The Se biofortification efficiency depends on a number of factors associated with the Se concentration in plants (also called bioavailability) during biofortification, such as plant species, Se species and source (chemical Se fertilizer, natural source of Se or organic Se), soil pH and redox conditions, soil organic matter, and the presence of competitive ions (Fordyce, 2007).

Plant species: Plants have been classified as hyperaccumulators (>1000 mg/kg, such as *Stanleya*), secondary accumulators (100–1000 mg/kg, such as Brassica species, broccoli), and non-accumulators depending upon Se accumulation inside their cells (Gupta & Gupta, 2017). Vegetables (e.g., Brassica species: pak choi and cabbage) normally accumulate more Se than legumes (beans), followed by cereals (wheat and rice). The Se concentration accumulated in fruits is generally low, whereas high concentrations (ranging from 0.03–512 mg/kg) have been reported in Brazil nuts as a result of natural biofortification (Prado *et al.*, 2017).

Se application methods: Different application methods of Se-based fertilizer affect Se accumulation and transformation in plants. Foliar application is generally more efficient in enhancing the Se concentration in plants in comparison with soil application (see Section 9.3). Studies showed that the efficiency of foliar Se application is on average eight times higher than soil application (Ros *et al.*, 2016). Besides, application of Se fertilizers at different plant growth stages can also result in a different biofortification efficiency.

Se species and source: The uptake rates and mechanisms of selenite, selenate and organic Se are different. Some studies showed that selenite is adsorbed and taken up in a faster passive way and readily reduced to organic compounds in plants, while selenate is taken up in an active way and easily distributed from roots to shoots (Arvy, 1993; Gupta & Gupta, 2017). Selenate reduction occurs via substitution for sulfate in the ATP sulfurylase reductase system, which is an ATP-consuming process and rate-limiting step, resulting in lower selenate accumulation in plants compared to selenite (Van Hoewyk, 2013). However, Ros *et al.* (2016) showed that biofortification using selenate-based fertilizers has a high potential to increase Se uptake by crops and subsequent Se intake by animals and humans. This is attributed to the fact that selenate is not easily adsorbed into the soil matrix in comparison with selenite, resulting in higher bioavailable Se

concentrations in the soil, while selenite is readily adsorbed in the soil environment (Ros *et al.*, 2016).

Soil pH and redox conditions: Soil pH and redox conditions have an important effect on Se availability since a combination of these factors determines the Se species present in a given soil environment. For instance, selenate is the predominant Se species in near-neutral pH environments under aerobic conditions, whereas selenite predominates at lower pH and redox potential. Selenate is much more mobile, and thus plant-available in soils than selenite which is tightly bound to positively charged binding sites in soils (Eich-Greatorex *et al.*, 2007). Besides, soil pH negatively correlates with the amount of Se adsorbed by soil (Li *et al.*, 2015b). Most studies have demonstrated that relatively high pH values in soil solutions lead to a higher Se accumulation by plants in comparison with low soil pH (Li *et al.*, 2016, 2017). This is attributed to the fact that soil with low pH contains a high amount of H^+ , which do not compete for positively charged binding sites with selenite/selenate in soil, thus leading to a relatively high bioavailable Se in the soil solution.

Soil organic matter: Organic matter influences Se availability in different ways. On the one hand, organic matter has a significant capacity to remove Se from the soil solution, and immobilize Se by both biotic and abiotic mechanisms, thus reducing Se bioavailability. On the other hand, organic matter can improve the soil structure and stimulate oxidizing conditions, thus enhancing Se bioavailability (Li *et al.*, 2017). The release of organic matter-immobilized Se through mineralization will increase the bioavailable Se concentration in a soil.

Competitive ions: The Se accumulation in plants can also be influenced by the presence of other ions, especially phosphate (PO_4^{3-}) and sulfate (SO_4^{2-}). Interactions between Se and those ions may occur in the soil or in the plant (Bingham, 1989). Li *et al.* (2008) studied the Se uptake in wheat under phosphorus and sulfur-starved conditions and demonstrated that selenite uptake is an active process mediated partly by phosphorus transporters. Likewise, the Se uptake can be negatively influenced by the addition of sulfur due to the chemical similarity between these two elements. Studies have demonstrated that selenate is taken up by sulfate transporters, thus the competition for the same transporters could inhibit Se uptake by plants when sulfur is applied (Li *et al.*, 2008). For instance, a decrease in the Se concentration in the shoots and roots of corn (*Zea mays*) was observed when the sulfur concentration in solution increased (Huang *et al.*, 2008). Supplementation of sulfur in the calcareous alluvial and yellow-brown soil reduces the Se contents in soybean (*Glycine max* L.) seeds (Deng *et al.*, 2021).

9.5 FUTURE PERSPECTIVES

Different biofortification strategies have been documented for improving human and animal dietary Se intake. However, an adequate comparison of these

approaches from economy, health, environment and social acceptance perspectives is still needed. The most cost-effective biofortification method with enhanced Se accumulation and crop yield as well as farmers' inclination as a function of crop growth conditions and the socio-economic environment should be selected. Microbial-assisted biofortification can play an important role in optimization of these biofortification strategies when aiming to simultaneously improve crop Se accumulation and crop yield. However, each biofortification practice must be carefully evaluated to prevent plant-derived food products from having toxic Se levels that may be harmful to the organisms feeding on them.

Additionally, specific issues related to each biofortification strategy should be further addressed. For instance, applying high quantities of Se fertilizers as soil or foliar application may not always be the most sustainable strategy, as this application can result in the leaching of excessive Se, thus requiring regular applications, which can make this approach more costly. Besides, the widespread use of Se for biofortification might cause Se contamination in the environment (e. g., water, soil and plant), which in turn poses potential threats for human and animal health. Selecting crops with a high ability to accumulate greater Se concentrations is needed in the conventional breeding approach, however the crop biodiversity and dietary diversity should also not be neglected. Genetic engineering is the most controversial method because of the fear of disturbing natural gene functions in food crops and potentially causing hazardous effects on humans and animals. Further research should, on the one hand, explore the specific genes contributing to higher Se accumulation and, on the other hand, assess the safety issues and tackle ethical barriers.

In terms of the application of Se-enriched organic materials into the soil, risk assessments should be carefully conducted to avoid other contaminants also ending up in the soil and edible products. Besides, more studies are still needed on the application of SeNPs as an emerging technology. The mechanism of SeNPs uptake from soil, as well as their translocation and transformation in higher plants need to be further unraveled. Understanding the effects of the SeNPs application on the soil microbial ecology is also necessary. Any unpredictable health effect arising from this strategy should be systematically evaluated, also involving the chemical modification and transformation of SeNPs during biofortification and food processing.

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