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Selenium transport and metabolism in plants: Phytoremediation and biofortification implications

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ABSTRACT

The aim of this review is to synthesize current knowledge of selenium (Se) transport and metabolism in plants, with a focus on implications for biofortification and phytoremediation. Selenium is a necessary human micro-nutrient, and around a billion people worldwide may be Se deficient. This can be ameliorated by Se biofortification of staple crops. Selenium is also a potential toxin at higher concentrations, and multiple environmental disasters over the past 50 years have been caused by Se pollution from agricultural and industrial sources. Phytoremediation by plants able to take up large amounts of Se is an important tool to combat pollution issues. Both biofortification and phytoremediation applications require a thorough understanding of how Se is taken up and metabolized by plants. Selenium uptake and translocation in plants are largely accomplished via sulfur (S) transport proteins. Current understanding of these transporters is reviewed here, and transporters that may be manipulated to improve Se uptake are discussed. Plant Se metabolism also largely follows the S metabolic pathway. This pathway is reviewed here, with special focus on genes that have been, or may be manipulated to reduce the accumulation of toxic metabolites or enhance the accumulation of nontoxic metabolites. Finally, unique aspects of Se transport and metabolism in Se hyperaccumulators are reviewed. Hyperaccumulators, which can accumulate Se at up to 1000 times higher concentrations than normal plants, present interesting specialized systems of Se transport and metabolism. Selenium hyperaccumulation mechanisms and potential applications of these mechanisms to biofortification and phytoremediation are presented.

1. Introduction

The element selenium (Se) is an essential factor in the structure of organic life. Many life forms, including humans, require Se in trace amounts because it is a necessary component of the amino acid selenocysteine (SeCys). Selenocysteine is often called the 21st proteinogenic amino acid due to its relative rarity—most species that require Se have between 1 and 30 proteins with a SeCys residue, typically in the active site (Zhang and Gladyshev, 2009). Humans have 25 known selenoproteins with a wide range of functions and consequent health effects. For example, the glutathione peroxidases function to scavenge free radicals and remove other oxidative damage-causing species (Rayman, 2012). The health effects of this class of selenoproteins include improved immune function, gastrointestinal and thyroid protection, and normal spermatogenesis (Rayman, 2012; Schmidt and Simonović, 2012). Although the functions of some human selenoproteins remain unknown (Regina et al., 2016), it is clear that selenoproteins on the whole are critical, because a complete lack of Se from the proteome is lethal (Bosl et al., 1997).

The United States Recommended Dietary Allowance for Se in adults is 55–75 μg/day (National Academy of Sciences, 2000). The amount of Se available for human consumption is ultimately dependent on the concentration of available Se in the local soil and the amount that edible plants are able to extract from this soil (Combs, 2001). Plants do not appear to require Se themselves (Zhang and Gladyshev, 2009), although in low concentrations Se has beneficial effects on plants including increased growth, antioxidative capacity, and resistance to biotic stresses (Pilon-Smits et al., 2009). In fact, any Se plants may contain is likely taken up non-specifically through the action of transport proteins for other nutrients, particularly sulfur (S) (Anderson, 1993). The Se content of human diets can vary widely, depending largely on the concentration of available Se in the soil of the region. Since Se-poor soils are found in populous regions worldwide (including parts of China, Siberia, Scandinavia, sub-Saharan Africa, and New Zealand), Se deficiency may affect as many as one billion people (Combs, 2001).

Selenium deficiency, like other micronutrient deficiencies, manifests
subtly. It is linked to increased susceptibility to infections (including COVID-19 and HIV), cancers (especially colorectal) and other diseases (Hoffmann and Berry, 2008; Zhang et al., 2020). There is also evidence that selenoprotein PHGPs (phospholipid hydroperoxidase glutathione peroxidase) is essential to sperm motility, and therefore male fertility (Ursini et al., 1999). Furthermore, Se deficiency can mimic the effects of iodine deficiency, leading to hypothyroidism (Arthur et al., 1992). The obscure yet debilitating effects of Se and other micronutrient deficiencies have earned them the collective appellation of “hidden hunger” (de Valença et al., 2017).

On the other end of the spectrum, selenosis—the condition caused by excessive Se—is as dangerous as Se deficiency. Chronic selenosis occurs when individuals ingest excess Se for long periods of time, at levels that are not high enough to cause immediate symptoms. The classic signs of chronic selenosis in humans are hair loss, nail damage, and rash. However, a range of other symptoms have been attributed to chronic selenosis, including various nervous and endocrine system effects, as well as increased risk of some cancers and type 2 diabetes (Vinceti et al., 2017). Though it is rarer than Se deficiency, there have been incidences of chronic selenium in recent history, such as the documented cases in Enshi, China between 1958 and 1963 (Hung et al., 2013) and in Punjab, India between 1997 and 1998 (Hira et al., 2004). Acute selenosis can also occur in humans, as in several cases stemming from the ingestion of Se-rich chemical products such as gun bluing agents and excessive quantities of dietary supplements. Such cases, depending on the dose of Se ingested, present symptoms ranging from those of chronic selenium to organ failure and death (MacFarquhar et al., 2010; Matoba et al., 1986; See et al., 2006).

Excess Se is dangerous to other animal species as well. Environmental Se contamination from industrial and agricultural activities has caused selenosis outbreaks in various species in recent decades. The release of Se into sensitive waterways is often the origin of the contamination. For example, during the 1980s in the San Joaquin Valley of California, USA, Se-rich drainage water from irrigated farms was diverted to maintain marshland in the Kesterson Reservoir. This marsh, part of the Pacific Flyway, served as a critical waterfowl wintering and nesting site. A study of birds collected there between 1983 and 1985 found that tissue Se concentrations were many times higher than normal, correlating to reduced body weight, feather loss, and other signs of poor physical condition. Moreover, many dead or moribund birds at the site were determined to have suffered from Se toxicity (Ohlendorf et al., 1990). In another case study in Western Colorado, USA, runoff from naturally Se-rich farmland over many years led to chronically elevated Se levels in the Colorado River, negatively affecting the survival of several native fish species (Hamilton, 1999). Similar stories of anthropogenic Se contamination continue to arise. Se contamination from both agricultural and oil refinery effluents has long been a cause for concern in the San Francisco Bay-Delta (Presser and Luoma, 2006). In a 2020 study of Se toxicity in minnows from the Bay-Delta area, at least 75% of minnows sampled demonstrated spinal deformities, which were linked to Se accumulation from both maternal tissues transferred to the offspring and feeding on contaminated prey (Johnson et al., 2020). Various mining industries also contribute to Se pollution, as for example when rainwater leaches Se from the exposed ash waste generated from coal-burning power plants (Khamkhash et al., 2017).

The effects of Se on ecological and human systems share an important commonality: plants can be strategically utilized to mitigate the degree to which Se impacts both systems. Human consumption of Se can be increased by planting crops which are bred or engineered to take up more Se from the soil. Similarly, planting Se-contaminated areas with species which can tolerate and accumulate nontoxic forms of Se can reduce the soil Se load, thereby mitigating the amount of Se runoff into waterways. Therefore, this review places the current understanding of plant Se biology into the context of these major issues. Section 2 covers Se uptake and translocation in plants and describes how this information can be used to breed Se-fortified crops to supplement diets in Se-poor communities. Section 3 describes plant Se metabolism, toxicity, and tolerance. In this section, metabolic reactions which produce detoxified selenocompounds are highlighted as potentially valuable mechanisms for the phytoremediation of Se-contaminated soils. Section 4 discusses Se hyperaccumulators (HAs), a class of plants which is able to accumulate and tolerate concentrations of Se approximately 1000 times higher than normal plants. Special attention is given to mechanisms of Se transport and metabolism which may enable this unique phenotype, because those mechanisms are instructive for both biofortification and phytoremediation purposes.

2. Selenium uptake and translocation in plants

Selenate (SeO₄²⁻) and selenite (SeO₃²⁻) are the two major forms of bioavailable Se in soil, with selenate predominating in oxic soils and selenite predominating in anoxic soils (White, 2018). Organic

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**Fig. 1.** Conceptual map of selenium transport in plants. Selenate (SeO₄²⁻) is highlighted in yellow, selenite (SeO₃²⁻) is highlighted in green, and the amino acids selenocysteine (SeCys) and selenomethionine (SeMet) are highlighted in blue. Transport of selenate via SULTR1;1 and SULTR1;2 is bolded because there is experimental evidence to support it (Barberon et al., 2008; Takahashi et al., 2000; Van Hoewyk et al., 2008). All other movement of selenocompounds is not bolded because the supporting evidence (discussed in Sections 2.1 and 2.2) is indirect and largely consists of interpretation from transcriptomic analyses. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
selenocompounds like selenoamino acids are also present in significant concentrations in some soils (Abrams et al., 1990; El Mehdawi et al., 2015a) and can be imported by plant roots (Kikkert and Berklelaar, 2013). Plants are capable of taking up both selenite and selenite ions at the root; however, neither ion is taken up through a Se-specific transporter. Selenate is taken up via sulfate transporters (SULTRs), and selenite is taken up by phosphate transporters and aquaporins (Schiavon and Pilon-Smits, 2017; White, 2018). Selenite is quickly metabolized upon uptake. Selenate, however, can be found throughout the plant (Huang et al., 2017). The conventional assumption is that various SULTRs are responsible for this distribution of selenate (Anderson, 1993). After discussing Se uptake in Section 2.1, Section 2.2 reviews selenate translocation to the various plant tissues. Note that, although there are no alternative theories for the transport of selenate, there is also no concrete evidence to support the hypothesis that SULTRs are solely responsible for selenate movement within the plant. Next, Section 2.3 discusses the plant Se transporters in terms of their biofortification and phytoremediation implications. Fig. 1 presents a visual synthesis of the known mechanisms of Se transport in a conceptual map.

2.1. Selenium uptake at the root

There are four groups of sulfate transporters in plants, all of which are H+/sulfate symporters (Gigdaghashvili and Kopriva, 2014). Group 1 SULTRs 1;1, 1;2, and 1;3 are high affinity transporters. SULTR1;1 and SULTR1;2 function to take up sulfate at the root (Yoshimoto et al., 2002), and studies in model species Arabidopsis thaliana have shown that both SULTRs are capable of transporting selenate as well. SULTR1;2 is the primary selenate and sulfate transporter, and SULTR1;1 takes up both ions to a significant but lesser extent (Barberon et al., 2008).

The expression levels of SULTR1;1 and SULTR1;2 are partially determined by the concentration of sulfate available at the roots. SULTR1;2 mRNA is approximately 10 times more highly expressed than SULTR1;1 in the root under normal S conditions (Rouached et al., 2008). Both SULTR genes are more highly expressed in the root under S deficiency conditions, although the increase in SULTR1;1 relative to control conditions is greater than that of SULTR1;2 (Rouached et al., 2008; Yoshimoto et al., 2002). Theoretically, increased expression of both SULTRs may also be expected when selenate is added to the substrate, because selenate competes with sulfate for binding to the SULTRs, effectively mimicking S starvation (Takahashi et al., 2000; Van Hoewyk et al., 2008). However, studies report conflicting results regarding SULTR1;1 and SULTR1;2 upregulation in A. thaliana plants exposed to selenate (Rouached et al., 2008; Takahashi et al., 2000; Zhang et al., 2006). The effect of selenate competition with sulfate thus remains unclear. Overall, SULTR1;1 and SULTR1;2 have somewhat overlapping functions, and the nature of their relationship and regulation is still undetermined. However, there is general agreement that SULTR1;2 is the predominant root sulfate and selenate uptake protein.

Although there is less research on the uptake of selenite, recent studies in rice (Oryza sativa) and tobacco (Nicotiana tabacum) indicate that inorganic phosphate (Pi) transporters may be capable of taking up selenite at the root (Song et al., 2017; Zhang et al., 2014). In both studies, overexpression of specific Pi transporters led to significantly greater selenite uptake from the substrate. There is also evidence that the aquaporin NIP2;1 is able to take up selenite in rice (Zhao et al., 2010). These mechanisms are both deserving of further study, because selenite is the prevalent form of Se available to plants, like rice, that are generally grown in anoxic soils (paddies). Understanding how plants take up selenite from anoxic soils will be useful for the engineering of crops in regions where dietary Se is limited.

Organic Se compounds, including selenoamino acids, can make up a significant proportion of the available soil Se in some soils (Abrams et al., 1990; El Mehdawi et al., 2015a). Plants are able to take up the amino acids selenocysteine (SeCys) and selenomethionine (SeMet) from the soil (Kikkert and Berklelaar, 2013), probably via root amino acid transporters with affinity for the S containing amino acids cysteine (Cys) and methionine (Met). A. thaliana amino acid permease (AAP) AAP1 and the AAP homolog LHT1 are broad-specificity amino acid transporters that have been shown to mediate uptake of Cys and Met (Boorer et al., 1996; Hirner et al., 2006). More research is required to experimentally determine whether these transporters take up selenoamino acids in addition to their S homologs.

2.2. Selenate translocation through the plant

2.2.1. Movement between organs

Several studies in wheat (Triticum aestivum) and rice indicate that, following uptake at the root, most selenate is translocated to the shoot (Huang et al., 2017; Li et al., 2008; Wang et al., 2015). It should be noted that similar studies of selenate distribution have not yet been performed in A. thaliana, but it is assumed here that the findings of such studies would be analogous to those in rice and wheat.

The low-affinity Group 2 SULTR2;1 and Group 3 SULTR3;5 are expressed in the xylem parenchyma and pericycle in A. thaliana and cooperate to load sulfate to the xylem (Kataoka et al., 2004a; Takahashi et al., 2000). SULTR2;1 appears to be the major transporter, while loss of SULTR3;5 somewhat reduces sulfate transport, loss of SULTR2;1 causes a much greater reduction of transport capacity (Kataoka et al., 2004a). Similar to the Group 1 SULTRs, SULTR2;1 expression appears to be inducible under S starvation conditions (El Mehdawi et al., 2018; Shimamichi et al., 2010) or when Se in the substrate is high (Takahashi et al., 2000). Conversely, SULTR3;5 expression is largely unaffected by S level (Kataoka et al., 2004a). Although there is no direct evidence, their functional similarity to the Group 1 SULTRs indicates that SULTR2;1 and SULTR3;5 are likely capable of transporting selenate as well.

Less is known about the redistribution of selenate and sulfate through the phloem. One early study indicated that high-affinity SULTR1;3 localizes to phloem companion cells in both roots and shoot, and plays a role in the mobilization of sulfate to sink organs (Yoshimoto et al., 2003). More recently, SULTR1;3 expression was shown to be upregulated in response to selenate treatment in wheat (Boldrin et al., 2016). Further studies are needed to elucidate the precise function of this transporter.

2.2.2. Intracellular movement

In the vacuole, Group 4 SULTR4;1 and SULTR4;2 both mediate sulfate efflux (Kataoka et al., 2004b). Transcription analyses in A. thaliana found that both SULTRs are more highly expressed in the shoot and are upregulated in response to high Se in the substrate (Zhang et al., 2006). This may be because competition with Se simulates S starvation, triggering export of stored sulfate from the vacuole to maintain S status. This would likely release stored selenate from the vacuole as well.

There are no known vacuolar sulfate importers. However, there are several pieces of evidence that together suggest a mechanism for influx of sulfate and selenate via anion channels. First, because the vacuolar lumen is positively charged relative to the cytosol (Martinioa et al., 2000), it is feasible that anions like selenate and sulfate could move passively into the vacuole down their electrochemical gradients via anion channels. Importantly, two parallel studies reported the discovery of channel-like transport proteins that mediate Pi influx to the vacuole (Liu et al., 2015, Liu et al., 2016). A much earlier study showed that Pi uptake into the vacuole was inhibited by competition with chromate, a sulfate analog (Missonneau et al., 2006). This indicates that sulfate (and thereby selenate) could move into the vacuole through these Pi transporters.

Sulfate uptake to the chloroplast is mediated by the five Group 3 SULTRs (3;1-3;5). Interestingly, chloroplast import constitutes a second distinct function for SULTR3;5, in addition to xylem loading (as discussed in Section 2.2.1). Studies of A. thaliana Group 3 SULTR mutants found that each of these SULTRs partially contributed to sulfate uptake (Cao et al., 2013; Chen et al., 2019). Interestingly, knockouts of any one
of the SULTRs 3;2, 3;3, or 3;4 demonstrated approximately 70% reduced sulfate uptake to the chloroplast. Knockouts of the other two SULTRs (3;1 and 3;5) caused significantly less sulfate uptake reduction. Regarding the overlapping contributions of the Group 3 SULTRs to sulfate uptake (especially 3;2, 3;3 and 3;4), the authors speculate that multiple different SULTRs may complex into a multimer, the function of which is lost or greatly reduced by the loss of any one subunit (Cao et al., 2013). This suggestion has not been experimentally supported. Regardless of the specific mechanism, it is likely that sulfate and selenate are taken up into the chloroplast by the Group 3 SULTRs.

There are no known chloroplast sulfate exporters. It is possible that sulfate and selenate move passively out of the chloroplast down their electrochemical gradients, because the overall charge in the chloroplast stroma is more negative than the cytosol (Pottosin and Shabala, 2016). However, to our knowledge, research into the existence of an anion channel or other transporter to export sulfate from the chloroplast has not been conducted to date. This may be because sulfate is assimilated into the S metabolic pathway in the chloroplast. Thus, mechanisms for export of S-containing metabolites, like the amino acids Cys and Met or the antioxidant glutathione (GSH), may be more physiologically relevant (Gigolashvili and Kopriva, 2014).

2.3. Biofortification and phytoremediation implications of selenium transport processes

The primary goal shared by Se biofortification and phytoremediation is to increase Se uptake from the soil. Without this foundation, subsequent goals such as improving plant Se tolerance or developing tissue-specific accumulation of selenoamino acids cannot be fully realized. Therefore, research to biofortify crops with Se or to remediate Se-rich soils should first focus on increasing Se uptake at the root. As discussed in Section 2.1, studies have already shown that both SULTR1;1 and SULTR1;2 are capable of transporting selenate in addition to sulfate. However, there has been little research into the effect of overexpression of these SULTRs on Se accumulation in crop species. A 2016 study demonstrated that tobacco plants overexpressing the soybean (Glycine max) SULTR1;2 homolog exhibited increased biomass and S content (Ding et al., 2016) — however, Se content of the transgenics and control plants were not reported. Similar studies could also analyze the effects on Se accumulation of overexpressing phosphate transporters, aquaporins like NIP2, and amino acid transporters. To our knowledge, no such studies have yet been performed.

3. Selenium metabolism, toxicity, and tolerance

Similarly to Se transport, Se metabolism in plants is predicted to largely follow the S pathway (White, 2018). However, there is significantly more experimental evidence to support this assertion for Se metabolism than for Se transport. Section 3.1 proceeds stepwise through the S assimilation pathway, discussing evidence indicating that Se assimilation follows the same route. Major branch points in the pathway are noted. Section 3.2 describes the specific mechanisms of Se toxicity, and Section 3.3 reviews the major methods of Se tolerance. Finally, Section 3.4 discusses significant questions of biofortification and phytoremediation which can be addressed by this research. Fig. 2 diagrams the known steps of the S and Se metabolic pathways.
3.1. Selenium metabolism

3.1.1. From selenate to selenocysteine

Although Se and S assimilation may occur in the root or shoot, most selenate taken up by the plant is transported to the shoot (Leustek, 1996; Pilon-Smits et al., 1999; Sors et al., 2005b). The S and Se assimilation pathways then split into cytosolic and plastidic branches. In the former, sulfate is activated to adenosine 5'-phosphosulfate (APS) by the cytosolic isomerase of the enzyme adenosine triphosphate sulfurylase (ATPS2) (Sick and Leustek, 1996; Bohrer et al., 2015b). Studies of yeast ATPS have shown that it acts on sulfate and also assimilates selenate to adenosine 5'-phosphoselenate (APSe) (Dilworth and Bandurski, 1977; Raspor et al., 2003). Furthermore, overexpression of ATPS1 in the plant species Brassica juncea and A. thaliana increased selenate reduction to APSe (Pilon-Smits et al., 1999; Sors et al., 2005a). In the next step, APS is phosphorylated to 3'-phosphoadenosine 5'-phosphosulfate (PAPS) by APS kinase (APK) (Bohrer et al., 2015a). PAPS serves as a donor of S for sulfated metabolites (Koprivova and Kopriva, 2016). It is not known whether APK also acts on APSe. More research is required to determine the fate of Se in the cytosol in plant shoots.

Significantly more research has been conducted to elucidate the plastidic branch of the S and Se assimilation pathways. Selenate, by analogy with sulfate, is probably able to enter the chloroplast of leaf cells via the Group 3 SULTRAs (Chen et al., 2019). In the chloroplast, sulfate and selenate are reduced to cysteine (Cys) and selenocysteine (SeCys), respectively (Sors et al., 2005b; White, 2018).

The first enzyme of this pathway is also ATPS, which has 4 plastidic isoforms. In A. thaliana, ATPS1, ATPS3 and ATPS4 localize only to the chloroplast, and ATPS2 is found in both the chloroplast and cytosol (Bohrer et al., 2015a, 2015b). In the plastid, ATPS activates sulfate and selenate to APS and APSe, respectively. Next, APS reductase reduces APS to sulfite (Seyta et al., 1996). Evidence indicates that this enzyme likely also acts to reduce APSe to selenite: a study of transgenic A. thaliana showed that overexpressing ATPS or APS reductase each increased the proportion of selenite in the plant, but that overexpressing both increased reduction to selenite still more highly (Sors et al., 2005a). This suggests that both enzymes work together, not only to reduce sulfate, but also selenate.

Sulfite and selenite are then further reduced to sulfide and selenide, respectively. The reduction of sulfite is performed by the enzyme sulfite reductase (SIR). However, it is not clear whether this enzyme is also capable of reducing selenite. Multiple studies have shown that inhibition or knockdown of SIR do not affect selenite reduction to selenide (Fisher et al., 2016; Ng and Anderson, 1979). It appears that reduction of selenite via an interaction with glutathione is more energetically favored (Hsieh and Ganther, 1975).

The Cysteine Synthase (CS) complex, established via a reversible complexation of O-acetylserine thiol lyase (OASTL) and serine acetyltransferase (SAT), incorporates sulfide into Cys (Bogdanova and Hell, 1997). This complex can be found in the mitochondria and cytosol in addition to the chloroplast (Heg et al., 2008). In vitro studies of this complex isolated from several plant species have confirmed that it is also capable of incorporating selenide into SeCys (Ng and Anderson, 1978). There are two major branches in the S/Se metabolic pathway following synthesis of Cys/SeCys. The first releases elemental Se from SeCys, and the second is the production of selenomethionine (SeMet), another selenoamino acid (Lima et al., 2018; White, 2018).

3.1.2. Release of elemental selenium from selenocysteine

Research in A. thaliana revealed that the chloroplast-localized cysteine desulfurase AtCpNifS is capable of both releasing elemental S from Cys and elemental Se from SeCys. Activity assays demonstrated that the enzyme more efficiently reacts with SeCys than Cys (Pilon-Smits et al., 2003). Elemental S released by AtCpNifS in the chloroplast may be used in the subsequent formation of Fe–S clusters, important cofactors for proteins involved in the photosynthetic electron transport chain (Balk and Lobléaux, 2005). It is possible that Se is subsequently misincorporated into Fe–Se clusters, damaging the photosynthetic apparatus. Interestingly, however, A. thaliana overexpressing AtCpNifS demonstrated overall increased tolerance to selenate (Van Hoewyk et al., 2005).

3.1.3. From selenocysteine to selenomethionine

In vitro studies of the enzyme cystathionine-gamma-synthase (CγS) from spinach (Spinacia oleracea) extract demonstrated that it is capable of synthesizing cystathionine (CγS) as well as selenocystathionine (SeCγS), from Cys and SeCys, respectively (Dawson and Anderson, 1988). Subsequently, CγS and SeCγS are split to form homocysteine (HCys) and selenohomocysteine (SeHCys), respectively, by cystathionine-beta-lyase (CβL). This step was again confirmed by in vitro analysis of crude plant extracts, including spinach extract (McCluskey et al., 1986).

Methionine synthase (MetH) catalyzes the final step of Met synthesis from HCys in plants. No experiments have yet shown that plant MetH is also capable of synthesizing selenomethionine (SeMet) from SeCys. However, mammalian MetH has been used to catalyze SeMet synthesis (Zhou et al., 2000). Both SeMet and Cys have long been known to accumulate in plants following Se treatment (Peterson and Butler, 1962). Therefore, it is most likely that plant MetH acts upon SeHCys as well. Up to this point, all steps of the Se metabolic pathway have occurred within the chloroplast. However, the localization of the final step in SeMet synthesis is unclear. Studies in A. thaliana have shown that MetH isoforms exist both in the cytosol (Ravanel et al., 1998) and the chloroplast (Ravanel et al., 2004).

3.1.4. Volatile product from selenomethionine

From Met and SeMet, plants can synthesize the volatile compounds dimethylsulfide (DMS) and dimethylselenide (DMSe), respectively. First, the enzyme S-adenosyl-L-Methionine-L-Methionine S-methyltransferase (MMT) methylates SeMet to Se-methyl selenomethionine (SeMM) (Tagmount et al., 2002). DMSe is then released from SeMM in one of two ways. In some plants, the enzyme methylmethionine hydrolase (MMH) cleaves SeMM and releases volatile DMSe directly (Lewis and Johnson, 1974). Alternatively, in other species SeMM may be converted to the intermediate dimethylselenoniuin propionate (DMSeP) (Tagmount et al., 2002). While there is no direct evidence for this, B. juncea plants supplied with DMSeP volatilized Se (as DMSe) at a rate six times that of plants supplied with SeMet (De Souza et al., 2000). This indicates that DMSeP is likely an intermediate that is quickly converted to volatile DMSe, via an unknown process.

3.2. Toxic selenocompounds and their effects

3.2.1. Selenocysteine and selenomethionine

Overall Se toxicity, as measured by reduction in dry weight productivity, is correlated to the ratio of Se to S concentrations in the shoot (White et al., 2004). This suggests that a major mechanism of Se toxicity is competition with S. This competition mainly manifests as the misincorporation of SeCys and SeMet in place of Cys and Met in protein synthesis.

Selenocysteine is thought to be the major source of problematic misincorporation into proteins (Van Hoewyk, 2013). Cys residues are often found at key locations in tertiary protein structures. For example, SeCys substitution can lead to the production of Fe–S clusters, important cofactors for proteins involved in the photosynthetic electron transport chain (Balk and Lobléaux, 2005). It is possible that Se is subsequently misincorporated into Fe–Se clusters, damaging the photosynthetic apparatus. Interestingly, however, A. thaliana overexpressing AtCpNifS demonstrated overall increased tolerance to selenate (Van Hoewyk et al., 2005).
clusters, which can damage critical photosynthetic processes (Van Hoewyk, 2013). For example, Stanleya albescens plants demonstrated photosynthetic rate reductions when grown on media with 20 μM selenate (Freeman et al., 2010).

SeMet is significantly less deleterious when incorporated into proteins than SeCys. The general toxicity mechanism of SeMet misincorporation stems from the fact that the rate of peptide bond formation between SeMet and subsequent amino acids is reduced relative to Met. Because Met is the amino acid encoded by the initiation codon, this can lead to an overall reduced rate of translation initiation, providing that the concentration of available SeMet is sufficiently high to compete with Met (Eustice et al., 1981; Van Hoewyk, 2013).

3.2.2. Selenate and selenite

Experimental evidence indicates that selenate exposure correlates strongly to increased accumulation of reactive oxygen species (ROS), which cause many types of physiological damage. Exposure to 20 μM Se as selenate via the growth media was sufficient to induce excess accumulation of the ROS superoxide and hydrogen peroxide in S. albescens plants (Freeman et al., 2010). A study of the effect of selenate on A. thaliana found that knocking out APS reductase (APR), involved in the reduction of selenate to selenite, causes a higher accumulation of ROS than in wild type, in selenate-exposed plants. In APR knockout plants, excess accumulation of selenate was found to correlate to reduced levels of GSH (Grant et al., 2011). This is problematic because it reduces the plant’s overall antioxidative capacity.

Selenate may be more toxic to plants than selenite. Multiple studies show that adverse effects of Se begin to appear at lower concentrations of selenite than selenate. In lettuce (Lactuca sativa) and cucumber (Cucumis sativus), biomass was found to decrease at lower concentrations of supplied selenite than selenate, and lipid peroxidation (free radical damage to lipids) increased significantly at lower selenite concentrations as well. Se concentrations were also higher in the roots of lettuce plants supplied with selenite, and higher in the shoots when supplied with selenite (Hawrylak-Nowak, 2013). All of the same results were also found in a later study of cucumber plants (Hawrylak-Nowak et al., 2015). The prevailing theory regarding the toxicity of selenite is that, whereas selenate is transported to the shoot and mostly remains as selenite, selenite is largely assimilated in the root into organic Se compounds like SeCys and SeMet, interfering with normal protein production (White, 2018).

3.3. Mechanisms of Se tolerance

Plants tolerate Se via a combination of several mechanisms. The major tolerance mechanisms appear to be GSH intervention and volatilization of DMSe. GSH has been reported to interact with selenate. This interaction produces oxidized GSH and reduces the level of selenate (Grant et al., 2011), which prevents some selenite from creating ROS, but also reduces overall levels of available GSH in the plant. Volatilization of DMSe also has been shown to significantly reduce the Se load of the plant (Lewis and Johnson, 1974).

Other mechanisms only moderately reduce Se toxicity, or only theoretically affect tolerance. For example, A. thaliana expresses protein homologous to the mammalian Se-binding proteins, and these may be involved in increasing Se tolerance (Agalau et al., 2005). However, the significance of this effect has not been studied. In addition, the action of AtCpNiFS can release elemental Se from SeCys, and overexpression of AtCpNiFS has been shown to increase Se tolerance (Van Hoewyk et al., 2005). While elemental Se is not toxic in itself, it may be misincorporated into Fe–Se clusters, so the net effect of this mechanism at higher levels of Se is unknown. Finally, a significant proportion of selenate is stored in the vacuoles of leaf cells—this selenate may be remobilized via the vacuolar S transport proteins SULTR4;1 and SULTR4;2 (Zhang et al., 2006).

3.4. Biofortification and phytoremediation implications of selenium metabolism

Overexpression of key enzymes in the Se metabolic pathway may improve plant species’ ability to accumulate larger quantities of Se without suffering toxic effects. Several enzymes have already been tested. Studies have demonstrated that both ATPS and APR are good candidates for overexpression to enhance reductive selenate assimilation. ATPS1 from A. thaliana overexpressed in B. juncea led to increased Se uptake and a greater proportion of Se reduced to nontoxic organic forms (Pilon-Smits et al., 1999). A later study of A. thaliana showed that plants overexpressing either endogenous ATPS1 or APR from the bacterium Pseudomonas aeruginosa reduced proportionally more selenate than control plants, and that plants constitutively expressing both enzymes were able to reduce still more (over 90%) selenite (Sors et al., 2005a). Furthermore, plants overexpressing ATPS1 had increased GSH levels (Pilon-Smits et al., 1999; Sors et al., 2005a), which should help the plant maintain proper redox status, and also may be involved in the reduction of ApSe to selenite (Dilworth and Bandurski, 1977). In field trials, a B. juncea transgenic line overexpressing A. thaliana ATPS1 was also found to accumulate over four times more Se than the wild type when grown on Se-contaminated soils (Banuelos et al., 2005).

The next enzyme in the pathway to SeCys synthesis is the Cysteine Synthase complex. The effect of overexpressing this complex on Se toxicity in plants has not yet been examined. Overexpression of the complex has been shown to increase Cys production and tolerance to toxic S-containing compounds in tobacco—however, the tolerance to Se has not been tested (Noji et al., 2001). Since Cys production was increased, toxic SeCys production would likely be increased as well. However, this could merit experimental analysis, especially if CpnNiFS is concurrently overexpressed. As mentioned in Section 3.1.2, AtCpNiFS catalyzes the release of elemental Se from SeCys at a much higher rate than S from Cys (Pilon-Smits et al., 2003; Van Hoewyk et al., 2005). Furthermore, although Se could be subsequently misincorporated into Fe–Se complexes, there is no evidence yet that this occurs to a sufficient extent that it has negative consequences for the plant (Van Hoewyk et al., 2005).

Enzymes involved in the production of SeMet from SeCys are also promising, because Se accumulated as SeMet is less toxic than SeCys (Van Hoewyk, 2013). The first enzyme in this pathway, cystathionine-gamma-synthase (CγS), has been tested. Transgenic B. juncea expressing A. thaliana CγS demonstrated increased volatilization of DMSe and increased tolerance to selenite, but not selenate (Van Huysen et al., 2003). Several species expressing A. thaliana CγS have also been shown to accumulate increased levels of Met (Amir, 2010). Overexpression of the next enzyme in the pathway, cystathionine-beta-lyase, has been shown to not significantly increase the amount of Met accumulated in potato (Solanum tuberosum) (Maimann et al., 2001). The final enzyme, methionine synthase (MetH), has not been tested. Nevertheless, the increase in Met accumulation and DMSe volatilization in plants overexpressing CγS indicates that this enzyme is more likely to be rate-limiting than CγL or MetH. CγS warrants further testing in other species of interest for phytoremediation.

It is unclear whether overexpressing enzymes involved in the synthesis of DMSe from SeMet increase the volatilization of Se. This is partly because research on these enzymes is limited to date. Overexpression in Escherichia coli of the first enzyme in this pathway, S-adenosyl-L-Methionine-Methionine S-methyltransferase (MMT), increased the synthesis of DMSe from SeMet increase the volatilization of DMSe tenfold compared to an untransformed control (Tagmount et al., 2002), but to our knowledge no similar study has been done in plants. Furthermore, no study has assessed the effect of overexpressing the subsequent enzyme, methylmethionine hydrolase. In some plants, it appears that the compound DMSeP is an intermediate between SeMM and DMSe. It has been shown that the production of DMSeP from SeMet is rate-limiting for Se volatilization, rather than the production of DMSe from DMSeP (De Souza et al., 2000). However, the
enzymes involved in both of these steps are still unknown and must be discovered before transgenic experiments can be performed.

4. Selenium hyperaccumulators

Many plant species experience reduced fitness when grown on soils with high concentrations of Se (Freeeman et al., 2010; Mikkelsen et al., 1989; Xue et al., 2001). However, some species have adapted to not only tolerate but thrive on high concentrations of Se. These species, called Se hyperaccumulators (HAS), preferentially take up Se from the soil and accumulate it to tissue concentrations reaching 1.5% of their dry weight, which is hundreds of times higher than adjacent vegetation (Cappa et al., 2014). There does not appear to be a Se toxicity threshold in HAS, nor are there any other demonstrated physiological or ecological fitness costs (Schiauvon and Pilon-Smits, 2017). The ability to hyperaccumulate Se benefits plant fitness in several ways. At low levels, Se accumulation improves antioxidant capacity, possibly by stimulating expression of stress response genes (Freeeman et al., 2010). At higher levels, ecological benefits emerge, including herbivore deterrent and toxicity (El Mehdi et al., 2015b; Galeas et al., 2008) and elemental allelopathy for surrounding vegetation (Schiauvon and Pilon-Smits, 2017). These species possess unique adaptations which allow for the accumulation and tolerance of such extreme levels of Se. Section 4.1 discusses the current state of knowledge regarding Se uptake and transport in HAS. Section 4.2 describes the differences between HA Se metabolism and normal Se metabolism, noting HA-specific mechanisms of Se tolerance. Section 4.3 briefly reviews other HA adaptations, including ecological benefits and plant stress response adaptations. Section 4.4 analyzes the potential applications of Se HAs for biofortification and phytoremediation. Fig. 2 includes metabolic processes unique to HAS.

4.1. Uptake and transport

Several lines of evidence indicate that HAS possess mechanisms to preferentially take up Se over S, or to translocate Se preferentially over S to shoot tissues. A study of 39 angiosperm species, comprising HA and non-HA species, found a larger Se:S ratio in the leaves of HAs (White et al., 2007). Leaf Se concentration was significantly higher in the HAs; sulfur levels were also higher in the HAs but not as elevated as Se. Another study, comparing HA Stanleya pinnata and related non-HA B. juncea, found that uptake of selenate was significantly less inhibited by the presence of competing sulfate for the HA than the non-HA, suggesting that the HA has at least one selenate-specific transporter (Harris et al., 2014). The underlying mechanism is uncertain, but a number of transporters may be involved in this process.

Recent transcriptomic analyses indicate that hyperaccumulators may regulate the expression of SULTRs differently than related non-HAs. For example, the HA S. pinnata has been demonstrated to constitutively upregulate several SULTRs, whereas non-HA sister species S. elata regulates these SULTRs with respect to the concentrations of supplied sulfate and selenate (Wang et al., 2018). Specifically, the HA constitutively expresses the root uptake protein SULTR1;2, root to shoot translocator SULTR2;1, phloem transporter SULTR1;3 (in the root only), chloroplast importer SULTR3;1 (in the shoot only), and vacuolar exporter SULTR4;1 (in the root only) (Wang et al., 2018). Another possible candidate for preferential root uptake is the amino acid transporter LHT1, which was hundreds of times more highly expressed in the roots of S. pinnata than S. elata (Wang et al., 2018). As discussed in Section 2.1, LHT1 is a broad-specificity amino acid transporter capable of taking up of Cys and Met (and therefore possibly SeCys and SeMet) in plant roots (Hirmer et al., 2006).

The form of supplied Se may also have a significant effect on the response of a HA. A recent transcriptomic analysis of the Se HA Cardamine huepinghanensis found that, when supplied with selenite, the HA in fact downregulated the expression of its SULTR1;2 homolog (Zhou et al., 2018). This result may be attributable to the fact that selenite is not likely to be transported through SULTRs, but rather through Pi transporters, due to the greater similarity of Pi to selenite (White, 2018). Interestingly, a recent study of the related HA C. violifolia found that supplied phosphate did not interfere with selenite uptake (Both et al., 2020), indicating that selenite might instead be taken up via aquaporins, as has been shown in rice with the aquaporin NIP1;2 (Zhou et al., 2010).

Hyperaccumulation is thought to have evolved independently many times (Cappa and Pilon-Smits, 2014); thus, different taxa may have evolved different and perhaps unique methods of Se accumulation. The best-studied HAS so far are Astragalus bisulcatus (Fabaceae) and Stanleya pinnata (Brassicaceae). In a transcriptomic analysis of multiple Se HA and non-HA species from the genus Astragalus, SULTR transcripts were generally found to be constitutively expressed across all species, regardless of supplied selenate or sulfate concentration (Cabannes et al., 2011). This was a surprising result, as previously the constitutive upregulation of various SULTRs had been considered a hallmark of Se hyperaccumulators.

SULTR1;2 is the only transporter, to our knowledge, that has been isolated and tested for sulfate and selenate transport affinities. SULTR1;2 homologs from the HA S. pinnata and non-HA S. elata were expressed in yeast, and overall Se and S accumulation in transformed yeast supplied with several concentrations of selenate and sulfate were compared. Overall, no conclusive evidence was found to support the contention that the HA SULTR1;2 had a greater specificity for selenate over sulfate, when compared to the non-HA SULTR1;2 (Guignardi, 2017). Similar studies should be undertaken to determine which, if any, SULTRs or other transport proteins demonstrate Se specificity in hyperaccumulator species. For example, in S. pinnata, the root to shoot translocator SULTR2;1 has been suggested as a likely candidate to test for Se-specific transport (Schiauvon et al., 2020).

4.2. Metabolism and tolerance

Hyperaccumulator and non-HA metabolism of Se differ in several ways, beginning at the first step, the reduction of selenate. Non-accumulators can reduce selenate via any of four ATPS isoforms (ATPS1–4). ATPS1, 3, and 4 localize to the plastid, and ATPS2 localizes to both the plastid and cytosol. The HA S. pinnata, on the other hand, expresses ATPS2 in the root 10 times higher than the other three isoforms (Wang et al., 2018). Furthermore, in this HA, ATPS2 only localizes to the cytosol (Jiang et al., 2018). In most plants, the majority of selenate is moved directly from the roots to the shoots—however, high concentrations of both ATPS2 transcripts and selenoamino acids have been found in S. pinnata roots, and ATPS2 expression is especially high in low-S conditions, with or without the presence of competing Se (Schiauvon and Pilon-Smits, 2017).

Some SeCys in HAs may be methylated to methylselenocysteine (MeSeCys) by the action of selenocysteine methyltransferase (SMT). Accumulation of this amino acid is not correlated to Se toxicity symptoms because it cannot be incorporated in place of Cys in polypeptides—thus, it does not interfere with normal protein synthesis (Neuhierl and Böck, 1996). SMT was first discovered in the hyperaccumulator Astragalus bisulcatus (Neuhierl and Böck, 1996). However, subsequent research indicates it is present in at least some non-HA species as well (Banuelos et al., 2007; Lyi et al., 2005). Non-hyperaccumulator species expressing transgenic SMT from HAs are also able to produce MeSeCys. Overexpression of the A. bisulcatus SMT in A. thaliana and B. juncea allowed both species to convert SeCys to MeSeCys, and to accumulate more Se overall (Ellis et al., 2004; LeDuc et al., 2004). Furthermore, in a study of several Astragalus species, SMT activity was on average six times higher in the HA species than the non-accumulators (Sors et al., 2005a). Hyperaccumulator S. pinnata also mainly accumulates Se as MeSeCys (Freeeman et al., 2006).

Finally, in addition to volatilizing Se as DMSe, hyperaccumulators are capable of volatilizing Se as dimethylselenide, DMDSe. This volatile compound is produced from MeSeCys. First, MeSeCys may be
converted to the methylselenocysteine selenoxide (MeSeCysSeO) intermediate (Arnold and Thompson, 1962) via an as-yet uncharacterized enzyme, or it may be converted directly to methaneselenol (MeSe) by cysteine sulfoxide lyase (CSL) (Hall and Smith, 1983). MeSe is subsequently converted to DMDSMe (Gabel-Jensen et al., 2010; Ranjard et al., 2002).

4.3. Other hyperaccumulator adaptations

The ability to accumulate high concentrations of Se confers a number of plant health benefits to Se HAs. Strong evidence indicates that Se HAs gain an ecological advantage in herbivore deterrence, without apparent deterrence of important pollinator species. HAs tend to concentrate Se in their epidermis (Freeman et al., 2006), which both sequesters it away from S metabolic processes and deters biotic attackers. There is also evidence that soil Se phytoenrichment from hyperaccumulator leaf litter acts as a form of elemental allelopathy by deterring the growth of Se-sensitive vegetation close to hyperaccumulator plants. These ecological adaptations have been reviewed extensively elsewhere (El Mehdawi and Pilon-Smits, 2012; Schiavon and Pilon-Smits, 2017).

Selenium HA species also appear to possess heightened stress response mechanisms as compared to normal plants. The stress defense hormones ethylene, jasmonic acid, and salicylic acid—which upregulate antioxidant mechanisms and biotic stress resistance processes—are constitutively expressed in the HA S. pinnata (Freeman et al., 2010; Wang et al., 2018). Overproduction of any of these hormones may also increase S and Se uptake and assimilation in hyperaccumulators (Wang et al., 2018). Several studies in non-HA species support the idea that upregulation of these defense hormones can provide protection from Se stress. For example, Se-resistant accessions of the non-HA A. thaliana were found to upregulate stress defense hormones in response to Se exposure (Tamaoki et al., 2008). In another study, B. juncea plants treated with toxic concentrations of Se demonstrated greater growth inhibition than plants treated with both Se and salicylic acid (Gupta and Gupta, 2016). Finally, a recent study found that overexpression of the ethylene response factor ERF96 in A. thaliana increases selenium resistance, through both increased antioxidative capacity and reduced Se uptake (Jiang et al., 2020).

Some HA species are capable of hyperaccumulating more than one element. One of the most well-studied HAs, Arabidopsis halleri, hyperaccumulates both zinc (Zn) and cadmium (Cd) (Fukuda et al., 2020). Another study species, Noccaea caerulescens, hyperaccumulates Zn, Cd, and nickel (Ni) (Rozhevnikova et al., 2020). The grass Pulicinella distans, a known boron (B) hyperaccumulator, has recently been found to also hyperaccumulate Se (Kök et al., 2020). This is, to our knowledge, the first multi-accumulator to hyperaccumulate Se. The selection pressures which give rise to multi-accumulators are not clear. A leading theory, the Joint Effects Hypothesis, states that accumulation of multiple toxic metals synergistically enhances a plant’s herbivory defense capability beyond the expected additive effects of the individual metals (Boyd, 2012).

4.4. Applications to biofortification and phytoremediation

The current understanding of Se transport in hyperaccumulators remains limited. It is clear that HAs regulate some Se transport proteins differently than non-HAs, and overexpress certain SULTR proteins, which may explain their high Se accumulation. However, no study has yet explained the high Se:S ratios characteristic of Se HAs. Therefore, Se HA transporters will benefit from significant further study before they are applied to biofortification or phytoremediation. In particular, identification of a selenate-specific transporter would be very beneficial for the creation of transgenics that can effectively take up Se in a high-S environment.

There are several HA enzymes involved in reductive Se metabolism that may also be overexpressed in species of interest for biofortification and phytoremediation. For example, hyperaccumulator S. pinnata appears to rely disproportionately on the isoform ATPS2 for selenate reduction to APSe (Jiang et al., 2018). This isoform may be tested in comparison to the primary isoforms used by non-HA species to determine whether HA ATPS2 can reduce selenite more effectively or has other unique properties.

Other promising enzymes for the improvement of phytoremediation and biofortification capabilities are those involved in the synthesis of MeSeCys and subsequent volatilization of DMDSMe. SMT has been demonstrated to be instrumental for plant accumulation of MeSeCys (LeDuc et al., 2004). The most common form of Se in HAs, MeSeCys is much less toxic than inorganic forms of Se and so is less of a concern when accumulated in plants, and may even be beneficial to consumers (Schiavon et al., 2020). In some species, MeSeCys can be converted to methaneselenol by CSL (Hall and Smith, 1983). Then, methaneselenol may be converted to DMDSMe by an unknown enzyme. If the rate-limited enzyme in this pathway were identified, it could be overexpressed in non-HA species, and Se volatilization could proceed via two routes (DMSe and DMDSMe)—which would significantly improve the capability of phytoremediation practices.

Though it is not the main focus of this review, it should be noted that overexpression of stress response-related genes is also a promising tool for Se biofortification and phytoremediation. Overexpression of genes that increase production of ethylene, jasmonic acid, or salicylic acid has been linked to increased Se tolerance in A. thaliana (Tamaoki et al., 2008). Improving Se tolerance is an important factor in the development of plant lines for phytoremediation; however, there are potential drawbacks of using stress hormones to achieve this goal. For example, although increased ethylene production has been shown to improve Se tolerance via increased antioxidative capacity, it can also repress the production of Se transport proteins and ultimately reduce overall Se uptake (Jiang et al., 2020).

Finally, it is worth noting that multi-accumulator species, such as P. distans, could be very useful for the phytoremediation of soils contaminated with multiple toxic elements. For example, a 2011 study compared ecotypes of S. pinnata to find salt and B tolerant lines which could remove Se from sediments from contaminated with salt, B, and Se (Freeman and Bañuelos, 2011). Now, however, an alternative for phytoremediation of these contaminated sediments has emerged: P. distans, which tolerates high salinity conditions and may be able to remove significant amounts of both B and Se from the sediment.

5. Conclusion

Improvements to Se phytoremediation and crop biofortification require a thorough knowledge of the processes of Se transport and metabolism in plants. Significant in this respect, however, is the fact that plants do not seem to require Se and thus do not have Se-specific pathways. Rather, Se is transported through the action of S transport proteins and metabolized via S metabolic enzymes. Therefore, understanding Se transport and metabolism in plants involves understanding the analogous S plant processes. This knowledge can then be applied to enhance Se uptake and assimilation to nontoxic forms in species of interest for Se phytoremediation or biofortification.

Enhancing Se uptake is likely achievable via overexpression of one or more SULTR proteins; this would be particularly effective if any of the HA SULTRs were found to have enhanced selenate-to-sulfate specificity. However, few experiments have been performed in this area to date. Comparatively more research has been conducted into the effects of overexpressing enzymes of the S metabolic pathway on Se accumulation. Transgenic plants overexpressing ATPS, SMT, and CpNiS have been shown to accumulate significantly more Se than wild-type plants. Transgenic B. juncea overexpressing the enzyme ATPS grew well (but slowly) on Se-rich soils, and accumulated over four times more Se in their leaves than wild type plants (Bañuelos et al., 2005, 2007).

When developing future studies, it should be noted that the most
effective strategies to increase Se accumulation and tolerance will likely combine increased uptake via overexpressed SULTRs with overexpression of enzymes in the Se metabolic pathway to increase the proportion of nontoxic selenocompounds in the plant. Fig. 3 suggests one such strategy. Sulfate transporters of interest (in addition to SULTR1;2) include SULTR2;1 and SULTR3;5 (which are likely responsible for root to shoot translocation of selenate), and the Group 3 SULTRs 3;2–3;4 (which are likely the main chloroplast selenate importers). Enzymes with phytoremediation and biofortification potential include ATS (part of the metabolic pathway from selenate to SeCys), CphNiS (which releases elemental Se from SeCys), CjS (part of the metabolic pathway from SeCys to SeMet), MMT (part of the metabolic pathway from SeMet to DMSe), and SMT and CSL (part of the metabolic pathway from SeCys to DMSeS). All of these are promising areas for future research.

CRediT authorship contribution statement

Richard C. Trippe III: Investigation, Writing - original draft, Writing - review & editing. Elizabeth A. H. Pilon-Smits: Writing - review & editing, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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