Summary

The importance of selenium (Se) for medicine, industry and the environment is increasingly apparent. Se is essential for many species, including humans, but toxic at elevated concentrations. Plant Se accumulation and volatilization may be applied in crop biofortification and phytoremediation. Topics covered here include beneficial and toxic effects of Se on plants, mechanisms of Se accumulation and tolerance in plants and algae, Se hyperaccumulation, and ecological and evolutionary aspects of these processes. Plant species differ in the concentration and forms of Se accumulated, Se partitioning at the whole-plant and tissue levels, and the capacity to distinguish Se from sulfur. Mechanisms of Se hyperaccumulation and its adaptive significance appear to involve constitutive up-regulation of sulfate/selenate uptake and assimilation, associated with elevated concentrations of defense-related hormones. Hyperaccumulation has evolved independently in at least three plant families, probably as an elemental defense mechanism and perhaps mediating elemental allelopathy. Elevated plant Se protects plants from generalist herbivores and pathogens, but also gives rise to the evolution of Se-resistant specialists. Plant Se accumulation affects ecological interactions with herbivores, pollinators, neighboring plants, and microbes. Hyperaccumulation tends to negatively affect Se-sensitive ecological partners while facilitating Se-resistant partners, potentially affecting species composition and Se cycling in seleniferous ecosystems.

I. Introduction

Selenium (Se) is arguably one of the most interesting elements for biology because it is both essential and toxic for most species, with a very narrow window between deficiency and toxicity compared with other trace elements (Stadtman, 1990). Another interesting property of Se is that it can exist in many oxidation states and in inorganic and organic forms, which can be transformed into each
other chemically or biochemically, either nonspecifically via sulfur (S) metabolic pathways (Se and S are chemically similar) or via Se-specific enzymes in organisms that require Se for their metabolism (Wilber, 1980; Brown & Shrift, 1982; Stadtman, 1990; Anderson, 1993; Mihara et al., 2006). Essential Se metabolism appears to be an ancient trait that is present in many prokaryotes and eukaryotes including algae, but was lost in plants and fungi, perhaps because Se was not readily available in terrestrial habitats (Zhang & Gladyshev, 2009). While not essential, Se is considered a beneficial element for plants, promoting plant growth and stress resistance (Hartikainen, 2005; Pilon-Smits et al., 2009).

Organisms that need Se do so because they contain a number of selenoproteins (25 in humans) that have selenocysteine in their active site (SeCys, also referred to as the 21st protein amino acid) (Zhang & Gladyshev, 2009). Incorporation of SeCys rather than Cys in the active site confers better redox activity to enzymes (Zhang & Gladyshev, 2009). The SeCys in selenoproteins is encoded by an opal (TGA) stop codon, which is recognized by a special transfer RNA (tRNA) as a triplet coding for SeCys because of a secondary structure in the adjacent mRNA that is termed the SeCys insertion sequence (SECIS) (Zhang & Gladyshev, 2009). The SeCys-tRNA initially binds serine, which is enzymatically converted to SeCys by modification of the hydroxyl (−OH) rest group to selenol (SeH) (Stadtman, 1990). Selenoproteins typically have redox functions; mammalian selenoproteins are involved in scavenging free radicals (cancer prevention), immune function (pathogen resistance), thyroid function and spermatogenesis (Rayman, 2012). The recommended daily Se intake for humans is 55 μg in the USA (National Institutes of Health; https://ods.od.nih.gov/factsheets/Selenium-HealthProfessional/#h2). One billion people worldwide have been estimated to be Se-deficient (Lyons et al., 2003), which often goes unnoticed and is an example of hidden hunger (Muthaya et al., 2013). Selenium deficiency in humans and livestock occurs in areas where soil Se concentrations are naturally low, including parts of China, northwestern Europe, Australia, New Zealand, sub-Saharan Africa, southern Brazil and parts of the USA (Oldfield, 2002). Most Se-deficient individuals worldwide have a mainly vegetarian diet, and thus depend on plants for their dietary Se intake, and therefore plant Se metabolism is important for human health. On the other side of the spectrum, Se toxicity in humans and livestock is a problem where soils are particularly rich in Se (seleniferous), such as in parts of the USA, Canada, China and India (Oldfield, 2002). When seleniferous soils or fossil fuels are used, Se can be released as a contaminant in agricultural and industrial wastewaters, typically in the form of selenate or selenite (Terry et al., 2000). Evaporation of wastewater streams in dry climates can exacerbate these problems; the most well-known example is the death and deformity of fish and migratory birds in the Kesterson Reservoir in California, USA (Ohlendorf et al., 1990). Various plant-based systems, both aquatic and terrestrial, have shown efficacy to clean up Se-polluted water or soil (Zhao & McGrath, 2009; Banuelos & Dhillon, 2010).

Plants can take up and assimilate inorganic forms of Se into SeCys and selenomethionine (SeMet) via S transporters and the S assimilation pathway (Anderson, 1993). When these seleno-amino acids are nonspecifically incorporated into proteins, replacing Cys and Met, this disrupts protein function and causes toxicity (Van Hoewyk, 2013). Several mechanisms have evolved to prevent Se toxicity, including reduction of selenate or selenite to insoluble elemental Se, methylation of SeCys and SeMet, and further conversion of these compounds to volatile dimethyl(di)selenide (DM(D)Se) (Shrift, 1969; Sors et al., 2005). At the global level, biological volatilization may contribute substantially to Se fluxes, and may even be responsible for the existence of certain seleniferous areas, such as in southeastern China (Blazina et al., 2014). Other seleniferous areas such as the western and central parts of North America probably result from oceanic sedimentation during the Cretaceous era when there was an internal seaway (Wilber, 1980). Se accumulation by plants may also constitute an important portal for Se into the food chain, and for global Se cycling (Winkel et al., 2015).

Different plant species show vastly different levels of Se accumulation, assimilation and volatilization (Zayed & Terry, 1992; White et al., 2007). This is interesting from an evolutionary and ecological perspective: what are the evolutionary benefits and constraints of having high levels of Se accumulation, assimilation and volatilization, and what are the ecological implications? The variation in Se physiology and biochemistry among plant species is also very relevant for human and animal nutrition, as well as environmental health. Plant capacity to accumulate, biotransform and volatilize Se may be harnessed to provide sufficient dietary Se in areas where Se deficiency is prevalent (biofortification) and also to clean up excess Se from seleniferous and polluted soils and wastewater (phytoremediation) (Zhao & McGrath, 2009). The two phytotechnologies can even be combined: cleaning up Se pollution while creating a nutritionally enriched crop (Banuelos & Dhillon, 2010). In the next sections, we will give an overview of Se uptake, metabolism and tolerance mechanisms in algae and higher plants, with special attention to Se hyperaccumulator species. Subsequent sections will focus on evolutionary and ecological aspects of plant Se accumulation.

II. Biochemistry and physiology of plant Se uptake, metabolism and tolerance

1. Algae

Selenium is essential not only for Bacteria and Archaea and animals, including humans (Novoselov et al., 2002; Rayman, 2012), but also for at least 33 species of microalgae belonging to six phyla. However, at high concentrations, Se can damage important metabolic processes in these organisms, such as respiration and photosynthesis (Geoffroy et al., 2007).

The two main chemical forms of Se occurring in aquatic environments are selenite (oxidation state 4+, or SeIV) and selenate (oxidation state 6+, or SeVI) (Fournier et al., 2010). The ability of algae to absorb selenate and selenite is a function of water pH over the range 5–9 (Riedel & Sanders, 1996; Tuzen & Sari, 2010). In the unicellular green microalga Chlamydomonas reinhardtii, selenate uptake is maximal when water pH is 8, whereas selenite uptake prevails under lower pH values (Riedel & Sanders, 1996).
The macronutrients S and phosphorus (P) can affect Se accumulation in algae, functioning as competitors of Se uptake (Lee & Wang, 2001; Fournier et al., 2010; Simmons & Emery, 2011). Specifically, S in the form of sulfate was reported to prevent selenate uptake in C. reinhardtii (Fournier et al., 2010; Simmons & Emery, 2011; Vriens et al., 2016), but not selenite transport in the same species and in the microalga Emiliania huxleyi (Araie & Shiraiva, 2009; Vriens et al., 2016). In C. reinhardtii, selenite is transported by a specific, saturable system at low concentrations and nonspecifically at higher concentrations (Morlon et al., 2006). While fluxes for selenite uptake are constant, selenate uptake rates decrease with increasing concentration, indicating the existence of a saturable transport system for selenate at high concentrations (binding affinity for substrate \(K_m\) = 89 μM) (Morlon et al., 2006; Vriens et al., 2016).

The coccolithophorid species E. huxleyi (Haptophytes), which has an obligatory growth requirement of nanomolar concentrations of Se (Obata et al., 2004; Araie & Shiraiva, 2009), exploits selenite as the predominant substrate for growth and uses two possible mechanisms to take it up: a high-affinity ATP-dependent active transport process \(K_m = 29.8\) nM, which is probably mediated by specific membrane transporters located on the cell surface, and a low-affinity passive transport process acting in the linear range (Obata et al., 2004).

The processes of Se uptake in macroalgae have gained attention only in recent years. The molecular mechanisms involved still remain to be elucidated and appear to be different from those reported in microalgae (Schiavon et al., 2012a, 2016). Elevated concentrations of selenite or selenate were shown to decrease Se influx in the macroalga Ulva australis, perhaps because of selenite and selenate transport system saturation, or repression of gene expression and/or activity of plasma membrane transporters (Schiavon et al., 2016). Furthermore, Se accumulation in thalli of Ulva sp. treated with selenate was not affected by the high sulfate concentration measured in sea water, therefore suggesting the existence of an S-independent selenate transport mechanism in this seaweed (Schiavon et al., 2012a).

Microalgae requiring Se can incorporate this nutrient in the form of SeCys into the structure of essential selenoproteins. Chlamydomonas reinhardtii uses selenoenzymes similar to those characterized in mammals (Novoselov et al., 2002). The complete selenoproteome recently identified in this species includes 12 selenoproteins representing 10 gene families (Grossman et al., 2007; Lobanov et al., 2007). Other unicellular algae using selenoproteins are Ostreococcus (Prasinophyceae), Cyanidioschyzon (Cyanidiales) and E. huxleyi (Haptophytes) (Maruyama et al., 2004; Palenik et al., 2007; Araie et al., 2008).

Among the selenoenzymes produced by microalgae, a thioredoxin reductase (TR) has been identified, which is a mammalian-type NADPH thioredoxin reductase containing a SeCys residue (Novoselov et al., 2002; Palenik et al., 2007; Araie et al., 2008). The increase of TR activity observed in Scenedesmus quadricauda cells exposed to a high Se concentration suggests a role for this enzyme in the stress response (Umysová et al., 2009). Furthermore, Se-induced toxicity was dependent on sulfate ion concentration, probably because selenate accesses the sulfate assimilation pathway to be converted into Se-amino acids, similar to what happens in plants. Recently, Se methylation leading to the production of the volatile Se compounds DMDSe and DMSe was discovered in C. reinhardtii, and production of DMDSe was found to be more prevalent compared with DMSe (Vriens et al., 2016). Because this volatilization process was very efficient in this alga, it might be inferred that microalgae may be responsible for a considerable portion of global biogenic Se emissions via production of methylated Se compounds that volatilize into the atmosphere (Winkel et al., 2015; Vriens et al., 2016). Precipitation may be an important source of soil Se, as shown by Blazina et al. (2014) for southern China; over the last 6.8 million years, Se distributions in these sediments were probably controlled by rainfall.

2. Plants – Se uptake

While there is no evidence for the essentiality of Se for higher plants, a number of beneficial effects of Se on plant physiology have been recognized in several plant species, as described in the next section (and reviewed by Pilon-Smits et al., 2009). As a consequence of the similarity of Se and S, the uptake, movement and metabolism of Se in plants is generally thought to mimic those of S (Anderson, 1993).

Plants mainly take up Se as selenate \(\text{SeO}_4^{2-}\) or selenite \(\text{SeO}_3^{2-}\). They also exhibit the capacity to absorb organic forms of Se such as selenocysteine (SeCys) and selenomethionine (SeMet), but not insoluble elemental Se \(\text{Se}^0\) or metal selenide compounds (White & Broadley, 2009). Generally, selenite is the most soluble and bioavailable form of Se for plants and is predominant in alkaline and well-oxidized soils. Conversely, selenite is primarily present in anaerobic soils or under aquatic conditions (Mikkelsen et al., 1989; Fordyce, 2005; White et al., 2007). Both selenate and selenite transport processes in plants are energy-driven (Lass & Ullrich-Eberius, 1984; Hawkesford et al., 1993; Terry et al., 2000; Sors et al., 2005; Li et al., 2008; Zhao et al., 2010). Selenite uptake by plants was initially thought to be a passive diffusion mechanism (Arvy, 1993), but more recent research with wheat \((Triticum aestivum)\) has shown that selenite transport is an active process because it can be prevented to a significant extent using metabolic inhibitors (Li et al., 2008; Zhang et al., 2014). The involvement of the phosphate transport system in the movement of selenite throughout a plant has been reported based on the evidence that increasing phosphate concentration reduced selenite uptake rates in different plant species (Broyer et al., 1972; Hopper & Parker, 1999), and P (inorganic phosphate (Pi)) limitation significantly enhanced selenite uptake in wheat (Li et al., 2008; Zhang et al., 2014). In rice \((Oryza sativa)\), the overexpression and knockout of Oryza sativa phosphate transporter 2, the most abundantly expressed Pi transporter in roots, resulted in a dramatic rise and decrease in selenite uptake, respectively (Zhang et al., 2014). These studies indicate that selenite and phosphate compete for uptake because they share a common transporter. Zhao et al. (2010) also suggested a role for silicon transporters in selenite absorption, as the silicon influx transporter Oryza sativa Nod26-like intrinsic proteins (OsNIP2;1) from rice was found to be permeable to selenite.

Compared with selenite, selenate uptake has been more widely studied. Selenate is taken up through a separate pathway from selenite transport, but increasing phosphate concentration reduced selenate uptake rates in different plant species (Zhang et al., 2014). The involvement of the phosphate transport system in the movement of selenate throughout a plant has been reported based on the evidence that increasing phosphate concentration reduced selenate uptake rates in different plant species (Zhang et al., 2014). The involvement of the phosphate transport system in the movement of selenate throughout a plant has been reported based on the evidence that increasing phosphate concentration reduced selenate uptake rates in different plant species (Zhang et al., 2014).
selenite. Because of its chemical similarity to sulfate, selenate enters plants using the root sulfate transport system (Terry et al., 2000; White et al., 2004, 2007; Sors et al., 2005; Shinmachi et al., 2010). The high-affinity sulfate transporters SULTR1;1 and SULTR1;2, which are involved in the primary uptake of sulfate from the rhizosphere, act as proton symporters; for one molecule of sulfate/selenate that is moved inside the root cells, three protons are also taken up (Lass & Ulrich-Eberius, 1984; Hawkesford et al., 1993). The expression of both transporters is controlled by the S status of a plant and by S available in the growth medium (Takahashi et al., 2011; Schiavon et al., 2015). SULTR1;1 is strongly up-regulated under S deficiency, while SULTR1;2 is less sensitive to S regulation and is constitutively expressed under both S-sufficient and S-deficient conditions (El Kassis et al., 2007; Takahashi et al., 2011). Both SULTR1 transporters exhibit the capacity to mediate selenate transport from the rhizosphere into root cells, as Arabidopsis thaliana sultr1;2 mutants are more tolerant to selenate than sultr1;1 mutants and wild-type plants, while sultr1;1-sultr1;2 double mutants show the highest selenate tolerance (Barberon et al., 2008). Selenate competes with sulfate for uptake by SULTR1 transporters, but also up-regulates SULTR expression, mimicking the sulfate deficiency response (Schiavon et al., 2012b). SULTR1;1 and SULTR1;2 display unequal functional redundancy, with SULTR1;2 playing a major role in selenate uptake by a plant (El Kassis et al., 2007; Barberon et al., 2008). The contribution of SULTR1;1 to selenate uptake is small under S replete conditions and only increases when S is limiting (El Kassis et al., 2007; White et al., 2007).

In addition to Se oxyanions, plants can take up organic forms of Se, probably via amino acid permeases, which are plasma membrane-localized carriers mediating the movement of amino acids in a cell (White & Broadley, 2009). The two most common forms of organic Se that can be absorbed by plants directly are the seleno-aminos acids SeCys and SeMet. In durum wheat (Triticum turgidum) and canola (Brassica napus), both SeCys and SeMet were taken up at rates over 20-fold higher than selenate or selenite (Zayed & Terry, 1992; Kikkert & Berkelaar, 2013).

3. Plants – Se assimilation and metabolism

Having been taken up by plants into the root symplast, selenate is probably further transported into the root xylem vasculature by group 2 low-affinity sulfate transporters (SULTR2;1 and SULTR2;2), aided by SULTR3;5, by analogy with sulfate (Kataoka et al., 2004). It can then enter leaf mesophyll cells (probably via type 1 SULTR transporters again), from where it enters chloroplasts, probably via SULTR3;1 (Cao et al., 2012).

By analogy with sulfate, selenate is thought to be able to access the S assimilation pathway where it is converted into Se-amino acids (Sors et al., 2005). The sulfate/selenate assimilation pathway is depicted in Fig. 1. First, selenate must be activated by the enzyme ATP sulfurylase (ATPS), which catalyzes the conversion of sulfate/selenate into adenosine phosphosulfate/selenate (APS/APSe) (Leustek, 1994; Sors et al., 2005). This step mainly occurs in plastids and has been reported to be rate limiting for Se assimilation as Brassica juncea transgenic plants overexpressing the isofrom ATPS1 and provided with selenate exhibited a higher capacity to accumulate organic Se compared with the wild type and were more tolerant to Se (Pilon-Smits et al., 1999). Such transgenics with enhanced Se accumulation may be applicable in biofortification or phytoremediation. Indeed, in the field, the ATPS1-overexpressing B. juncea plants proved four to five times more effective in

**Fig. 1** Schematic model of selenium (Se) metabolism in plant mesophyll cells. Red text and arrows indicate Se hyperaccumulator processes. Asterisks indicate enzymes overexpressed via genetic engineering. Sultr, sulfate/selenate cotransporter; APSε, adenosine phosphoselenate; APS, adenosine phosphosulfate; GSH, glutathione; SAT, serine acetyltransferase; OAS, O-acetylselenere; (Se) Cys, (seleno)cysteine; OPH, O-phosphohomoserine; (Se)Met, (seleno) methionine; MMT, methylmethionine methyltransferase; DMSeP, dimethylselenopropionate; DM(OD)Se, dimethyl(0) selenide (volatile); SMT, selenocysteine methyltransferase.
accumulating Se from contaminated sediments (Bañuelos et al., 2005).

To date, four ATPS isoforms have been identified in A. thaliana, all localized to the plastids (Anjum et al., 2015). The isoform ATPS2 was shown to have a dual localization, both plastidic and cytosolic (Bohret et al., 2015). The APSe formed in the reaction mediated by ATPS is further reduced to selenide in the plastids by the activity of APS reductase (APR). The enzyme APR has been reported to be of crucial importance for the control of selenate assimilation in A. thaliana (Suter et al., 2000; Sors et al., 2005). In A. thaliana transgenics overexpressing APR, for instance, increases were observed of both Se flux through the plant and selenate reduction into organic forms (Sors et al., 2005). Also, A. thaliana apr2-1 mutants showed enhanced accumulation of selenate, but decreased production of selenite, indicating that APR2 is involved in APSe conversion to selenite (Grant et al., 2011). The same mutants displayed low tolerance to selenate because of reduced concentrations of glutathione (GSH), a key molecule involved in preventing and reducing oxidative stress (Grant et al., 2011).

In the next step of Se assimilation, selenite is reduced to selenide (Se\(^{2-}\)), which is then incorporated into SeCys (Terry et al., 2000; Sors et al., 2005). Selenite reduction to selenide may happen through two possible mechanisms. In the first, selenite may be converted enzymatically to selenide by sulfite reductase (Sir) (White, 2016), which also catalyzes the conversion of sulfite to sulfide (Yarmolinsky et al., 2012). The reduction of selenite to selenide may also occur as a result of an interaction between selenite and reduced GSH (Terry et al., 2000; Anderson & Mcmahon, 2001). In this case, selenite and GSH are initially converted nonenzymatically to selenodiglutathione (GSSeG), which is further converted to selenopersulfide (GSSeH) and finally to selenide through the action of the enzyme glutathione reductase (GR). GR usually mediates the reduction of GSH from its oxidized state (GSSG) in the chloroplasts using NADPH as an electron donor. In support of the GSH-mediated reduction of selenite, GSH from yeast was shown to reduce selenite into selenide (Hsieh & Ganther, 1975).

The synthesis of SeCys from O-acetylserine (OAS) and selenide probably can occur in the plastoplasts, cytosol and mitochondria via O-acetylserine thiol lyase (OASTL), which forms a cysteine synthase (CS) complex together with serine acetyltransferase (SAT), the enzyme that produces OAS (Wirtz & Hell, 2006; Pilon-Smiths, 2012; White, 2016), in analogy with the formation of Cys (Giovanelli, 1990). The CS complex (and, consequently, S/Se assimilation) is subject to positive regulation by OAS and negative regulation by sulfate or sulfide (Wirtz & Hell, 2006). Other factors involved in S-deficiency responses are the transcription factor SLIM transcription factor and microRNA395 (miRNA395) (Kawashima et al., 2009, 2011).

The amino acid SeMet can be produced from SeCys in a three-step enzymatic process (Fig. 1). Initially, SeCys is converted to Se-cystathionine through the condensation of O-phosphohomoserine (OPH) and SeCys, which is catalyzed by cystathionine-\(\gamma\)-synthase (CGS) (Sors et al., 2005; Pilon-Smiths, 2012). This reaction represents a rate-limiting step in the conversion of SeCys to volatile DMSe, a mechanism that plants may use to alleviate Se toxicity (Van Huysen et al., 2003). Brassica juncea transgenics overexpressing CGS exhibited higher Se volatilization rates, decreased Se concentration in leaf and root and enhanced Se tolerance compared with the wild type (Van Huysen et al., 2003, 2004). Such plants with enhanced Se volatilization are attractive for Se phytoremediation, as they may remove the pollutant from the site without the need for harvesting the plants (Terry et al., 2000). Se-cystathionine may be converted to Se-homocysteine via a reaction mediated by cystathionine beta-lyase (CBL). This enzyme has been shown to have the capacity to cleave both Se-cystathionine and cystathionine into Se-homocysteine and homocysteine, respectively, in various plant species (Sors et al., 2005). Finally, Se-homocysteine may be converted to SeMet in a reaction catalyzed by the enzyme methionine (Met) synthase, which uses methyl-tetrahydrofolate as a carbon donor (Cossins & Chen, 1998).

The misincorporation of SeCys and SeMet into proteins, instead of Cys and Met, is thought to represent an important cause of Se toxicity in plants, as it disrupts correct protein folding, leading to loss of function (Stadtmann, 1990; Pilon-Smiths, 2012). Perhaps as a detoxification mechanism, plants can volatilize Se in the form of DMSe using SeMet as a precursor (Tagmount et al., 2002). The first step in this Se volatilization from SeMet involves the methylation of SeMet to form methyl Se-Met (SeMM) by the enzyme S-adenosyl-l-Met:Met-S-methyltransferase (MMT) (Tagmount et al., 2002). SeMM can be directly converted to DMSe by the enzyme methylmethionine hydrolase, or via the synthesis of the intermediate molecule 3-dimethylselenoniopropionate (DMSeP) (Ellis & Salt, 2003). Aside from potentially decreasing toxic Se concentrations in plants, Se volatilization may play ecological roles, as described in section IV.

Other S compounds that Se may be incorporated into include (seleno)glutathione (Lindblom et al., 2013) and glucosinolates (Matich et al., 2012, 2015). Se may also be incorporated into iron (Fe)-Se clusters, as the enzyme that releases elemental S from Cys for the formation of FeS clusters can also utilize SeCys as a substrate (Van Hoewyk et al., 2005). Overexpression of this (SeCys lyase) enzyme led to reduced incorporation of Se into protein and enhanced Se tolerance and accumulation, indicating that the elemental Se produced does not cause toxicity (Van Hoewyk et al., 2005). The SeCys lyase-overexpressing SL plants accumulated twofold more Se, both in lab studies and in a field phytoremediation experiment, which may be useful for Se biofortification or phytoremediation (Bañuelos et al., 2007).

4. Molecular Se responses and tolerance

When supplied to plants at low concentrations (up to a few micromolar), Se tends to have a beneficial effect on plant growth for many plant species (Lyons et al., 2003; Cartes et al., 2005; Hartikainen, 2005; Pilon-Smiths et al., 2009). Similar growth stimulation has also been found for other toxic elements, and is termed hormesis (Poschenrieder et al., 2013). While essential Se metabolism is thought to have been lost in higher plants, based on in silico analysis of plant genomes that showed no evidence of a SEClS (Zhang & Gladyshev, 2009), it is feasible that Se is incorporated nonspecifically into a functional metabolite that
benefits a plant. It is also possible that low concentrations of a toxic element such as Se up-regulate plant pathways involved in stress resistance, thus priming a plant for adverse conditions. Se, even at low concentrations, has been found to up-regulate enzymes involved in antioxidation reactions, such as peroxidases and reductases (Pilon-Smits et al., 2009). Moreover, treatment with Se has been found to protect plants from several abiotic stresses including ultraviolet light, arsenic and heavy metal stress (as reviewed by Feng et al., 2013). As described in more detail in a following section, Se treatment also protects plants from biotic stresses, including pathogen attack and herbivory. This protection typically requires higher Se concentrations than those promoting growth, and is probably attributable more to a direct toxic effect of Se on the pathogen or herbivore than to up-regulation of defense pathways.

At high Se concentrations, starting at c. 20 micromolar depending on the plant species, Se has a negative effect on plant growth and causes chlorosis, particularly in the younger leaves. One reason for this toxicity may be that selenate and selenite at high concentrations cause oxidative stress in plants (Van Hoewyk, 2013). Additionally, Se can nonspecifically replace S in S compounds, including proteins (Van Hoewyk, 2013). Indeed, more ubiquinated proteins (i.e. damaged proteins, targeted for degradation) were found in Se-treated plants (Van Hoewyk, 2013). Se-related chlorosis in young leaves may also suggest S deficiency. In this context, it is interesting that the transcriptome responses of A. thaliana to Se were similar to those observed in response to S deficiency, and include up-regulation of genes involved in sulfate transport and assimilation (Van Hoewyk et al., 2008). This response to Se is expected to result in higher concentrations of S (and Se), which were indeed observed, both for total S and for the reduced antioxidant S compound GSH, which may help a plant tolerate Se (Van Hoewyk et al., 2008).

Plants can also avoid SeCys incorporation into proteins by either breaking down SeCys into elemental Se and alanine (Van Hoewyk et al., 2005) or by converting it to volatile DMSe (Terry et al., 2000). In agreement with a role of S assimilation in Se tolerance, Zhang et al. (2006b) identified an A. thaliana chromosomal region correlating with selenate resistance via quantitative trait locus (QTL) analysis, which included several genes involved in sulfate/selenate uptake and assimilation. Furthermore, a macroarray study comparing the expression levels of c. 350 genes between an S-resistant and an S-sensitive accession of A. thaliana grown in the presence or absence of Se found that Se treatment resulted in up-regulation of genes involved in sulfate/selenate uptake and assimilation as well as antioxidative pathways, and that the S-resistant accession had constitutively higher expression levels of these genes than the S-sensitive accession (Tamaoki et al., 2008a). Furthermore, the plant hormones jasmonic acid (JA) and ethylene appeared to be present at higher endogenous concentrations in the more S-resistant A. thaliana accession, and to be up-regulated by Se (Tamaoki et al., 2008a). Supposing these hormones to S-sensitive accessions rendered them more Se-resistant, and mutants or transgenics with enhanced or reduced levels or signaling of these hormones showed corresponding S resistance (Tamaoki et al., 2008a,b). Thus, Se resistance and accumulation in A. thaliana appear to be associated with enhanced levels of sulfate/selenate uptake and assimilation, which may be regulated via higher concentrations of JA and ethylene (for a model see Fig. 2 of Tamaoki et al., 2008b).

5. Hyperaccumulators

Depending on the capacity of species to accumulate Se in their natural habitat, three classes of plants are generally distinguished: Se nonaccumulators (< 100 mg Se kg\(^{-1}\) DW), secondary Se accumulators (100–1000 mg Se kg\(^{-1}\) DW) and hyperaccumulators (> 1000 mg Se kg\(^{-1}\) DW) (Brown & Shrift, 1982; Anderson, 1993). Hyperaccumulation of Se has been described for 45 taxa in six families (Cappa & Pilon-Smits, 2014; White, 2016). The genus *Astragalus* (milkvetch, Fabaceae) harbors most (25) Se hyperaccumulators; other well-documented hyperaccumulators have been found in the genera *Stanleya* (Prince’s plume, Brassicaceae), *Oonopsis* (goldenweed, Asteraeaceae), *Xylorhiza* (woodyaster, Asteraceae) and *Symphyotrichum* (white heath aster, Asteraceae) (Rosenfeld & Beath, 1964; El Mehdawi et al., 2014). These hyperaccumulator species are all perennial forbs that predominantly occur on seleniferous soils in the western USA where they may accumulate Se to 1.5% of DW (Galeas et al., 2007). Considering the distribution of Se hyperaccumulation across the plant kingdom, it is likely that Se hyperaccumulation has arisen independently in different plant families, and therefore different taxa may have evolved different hyperaccumulation mechanisms. Yet, hyperaccumulators from different families show many similarities.

Hyperaccumulators distinguish themselves from other species by their 10–100× higher Se concentrations as well as higher tissue Se : S ratios (White et al., 2007). The tissue Se : S ratios in hyperaccumulators are also higher than that of their growth substrate, which suggests that they have a transporter with a preference for selenate over sulfate (Cappa et al., 2014; Harris et al., 2014). Furthermore, hyperaccumulators contain Se almost exclusively in organic forms, predominantly as methyl-SeCys and sometimes also some γ-glutamyl-methyl-SeCys (in *Astragalus*) or selenocystathionine (in *Stanleya*), while nonhyperaccumulators accumulate more inorganic Se (Pilon-Smits et al., 1999; Pickering et al., 2003; Freeman et al., 2006b). This suggests that hyperaccumulators have a more active sulfate/selenate assimilation pathway. Selenium partitioning across different plant organs is also different for hyperaccumulators: they have higher shoot : root Se ratios as well as higher source : sink Se ratios in xylem and phloem as compared with nonhyperaccumulators (White et al., 2007; Cappa et al., 2014). Moreover, hyperaccumulators show patterns of spatial and temporal Se sequestration that are different from S patterns and also different from those of nonaccumulators (Galeas et al., 2007; Quinn et al., 2011). Hyperaccumulators particularly sequester Se in their epidermis, including leaf hairs, where it may serve a defensive function, and also is kept away from sensitive metabolic processes and thus may help prevent Se toxicity (Freeman et al., 2006b). Indeed, hyperaccumulators do not show Se toxicity symptoms, even when the plants contain levels of Se that are more than 1% of their dry
weight; they are truly Se hypertolerant. As a final distinction, it is interesting to note that the positive growth response of plants to Se is much more pronounced for hyperaccumulators, which may reach twice as much biomass when supplied with Se (El Mehdawi et al., 2012).

Transcriptomic and biochemical investigations into the mechanisms of Se hyperaccumulation in Stanleya pinnata and Astragalus bisulcatus have revealed that these hyperaccumulators have constitutive high expression of several SULTR selenate/sulfate transporters that probably mediate selenate uptake and translocation (Freeman et al., 2010; Cabannes et al., 2011; Schiavon et al., 2015). SULTR1 transporters not only appear to be constitutively and highly expressed in the roots of Se hyperaccumulator species, but SULTR1:2 may also have a substrate preference for selenate over sulfate, which may explain the extraordinarily high Se : S ratios in their tissues (Freeman et al., 2010; Cabannes et al., 2011; Schiavon et al., 2015; White, 2016). The preferential uptake of selenate over sulfate in Se-hyperaccumulators could be ascribed to differences in the amino acid sequences between SULTR1 isoforms identified in Se hyperaccumulators and nonhyperaccumulators. SULTR1 isolated from several hyperaccumulator species belonging to the genus Astragalus (Fabaceae) contain an alanine residue instead of the glycine typical of SULTR1 isoforms of nonaccumulating angiosperms (Cabannes et al., 2011; White, 2016). Compared with the sulfate transporter homologs in Se nonhyperaccumulators, SULTR1:1 and SULTR1:2 in Se hyperaccumulators appear to be less sensitive to regulation by plant S status and S supply (Schiavon et al., 2015). In consideration of this, SULTR1 transporters from Se hyperaccumulators, especially SULTR1:2, may be an important target for genetic engineering aiming to generate plants that accumulate high concentrations of Se even in high-S habitats, for biofortification and phytoremediation purposes.

Transcriptomic comparison of hyperaccumulator S. pinnata with nonhyperaccumulator relatives also showed enhanced transcript levels of several enzymes in the sulfate/selenate assimilation pathway (ATPS, APR and CS), which may explain why they accumulate more organic Se (Freeman et al., 2010; Schiavon et al., 2015). Enhanced conversion of inorganic to organic Se is thought to cause more oxidative stress (Van Hoewyk, 2013). S. pinnata hyperaccumulators and nonaccumulators did not show differences in activity levels of sulfate reductive assimilation enzymes (Sors et al., 2005). While these enzymes are probably important for selenate reduction in A. bisulcatus, the flux through the selenate assimilation pathway may be more controlled by sink depletion in this species (conversion of Cys to other forms of organic Se).

Organic forms of Se can also cause toxicity, particularly SeCys if it is nonspecifically incorporated into proteins (Brown & Shrift, 1982; Stadtman, 1990). Hyperaccumulators use the plastidic enzyme SeCys methyltransferase (SMT) to convert SeCys to methyl-SeCys, thus avoiding this type of toxicity (Fig. 1; Brown & Shrift, 1982; Neuhierl & Böck, 1996; Sors et al., 2009). The importance of SMT for plant Se tolerance was confirmed when an A. bisulcatus SMT was expressed in B. juncea: the transgenics accumulated more Se, which was in the form of methyl-SeCys, and were more Se tolerant (LeDuc et al., 2004). While SMT activity is particularly high in hyperaccumulators, it is not unique for this class of plants, as it was also found in broccoli (Brassica oleracea) (Lyi et al., 2005). Other potential Se tolerance mechanisms in hyperaccumulators are sequestration of methyl-SeCys in epidermal tissues (Sors et al., 2009; Freeman et al., 2010) and conversion to volatile DMDSe (Terry et al., 2000; Sors et al., 2005).

In summary, qualities that make Se hyperaccumulators different from nonhyperaccumulator species are not only their capacity to accumulate high concentrations of Se in their tissues, but also their capacity to take up selenate preferentially over sulfate, to more effectively assimilate inorganic Se and convert it to methyl-SeCys, to translocate Se independently from S via xylem and phloem, to store it preferentially in epidermal structures in young leaves and in pollen, ovules and seeds in reproductive organs, and to volatilize it as DMDSe. The mechanisms underlying these physiological traits, as judged from transcriptomic and biochemical analyses of S. pinnata in comparison with nonhyperaccumulator relatives, appear to be constitutively elevated expression levels of genes involved in sulfate/selenate uptake and assimilation as well as genes involved in production of antioxidants; genes involved in defense against biotic stresses were also up-regulated but their function in Se tolerance or accumulation is not clear (Freeman et al., 2010). In the same study, it was found that genes involved in the production or signaling of the stress/defense hormones JA, salicylic acid (SA) and ethylene were constitutively up-regulated in the hyperaccumulator (Freeman et al., 2010). These hormones have been reported to up-regulate sulfate uptake and assimilation, as well as antioxidation-related genes and biotic stress-related genes (Sasaki-Sekimoto et al., 2005). The hyperaccumulator S. pinnata may be continuously in a state of biotic stress alert, leading to elevated concentrations of JA, SA and ethylene. These hormones in turn up-regulate sulfate/selenate uptake and assimilation, oxidative stress resistance mechanisms and biotic defense proteins. These effects together lead to enhanced Se tolerance and accumulation. More studies are needed to better understand why S. pinnata would continually sense biotic attack; perhaps a receptor is deregulated. It is interesting to note the similarities between Se responses and tolerance mechanisms in the nonaccumulator A. thaliana and the related Se hyperaccumulator S. pinnata (Fig. 2).

III. Evolutionary aspects of plant Se (hyper) accumulation

1. Evolutionary benefits and constraints of Se accumulation

Based on its taxonomic distribution across many plant families, it is likely that Se hyperaccumulation is a derived trait and has evolved independently at least six times (Cappa & Pilon-Smits, 2014). If the evolution of Se hyperaccumulation in different lineages was independent, it may have been driven by the same or different selection pressures. Natural selection needs natural variation in a trait for it to act upon, which indeed appears to be there for Se tolerance and accumulation. Within species, different populations
can vary genetically in their Se tolerance and their (hyper) accumulation capacity, which may reflect adaption (Feist & Parker, 2001; Zhang et al., 2006a; El Mehdawi et al., 2015b). Within a hyperaccumulator field population, Se concentrations can also differ substantially, by at least 10-fold (Beath et al., 2004). At more elevated tissue concentrations, Se protects plants against a wide variety of invertebrate and vertebrate herbivores as well as certain fungal pathogens, and may also offer allelopathic benefits, as described in the next section (El Mehdawi & Pilon-Smits, 2012). Thus, it is feasible that over time there has been continuous selection for higher and higher Se accumulation capacity, through increasing ecological benefits. Another hypothesis is that Se hyperaccumulation is inadvertent: higher Se concentrations are generally associated with higher S concentrations, which may actually be under positive selection. In the different stages of evolution from a nonaccumulator via a secondary accumulator to a hyperaccumulator, any or all of these selection pressures may have played a role in different plant lineages.

There may also be evolutionary constraints on Se hyperaccumulation. Any physiological constraints resulting from toxicity appear to have been overcome through the evolution of Se tolerance mechanisms (Quinn et al., 2011). Perhaps there is a growth cost associated with Se hyperaccumulation; this has not been well studied. It might also be hypothesized that the toxic Se concentrations in hyperaccumulators have a negative effect on mutualist symbionts. However, studies so far show no evidence for this. Pollinators are not deterred by high floral Se concentrations (Quinn et al., 2011), and hyperaccumulators are colonized by an equally species-rich endophyte microbiome (Sura-de Jong et al., 2015). More studies are needed to better understand all the benefits and potential costs of Se (hyper)accumulation in different habitats.

2. Evolution of Se hyperaccumulation in Brassicaceae

To obtain better insights into the evolution of Se hyperaccumulation in a plant lineage, the Brassicaceae may serve as a case study. In this family, the hyperaccumulator S. pinnata may have evolved from a nonaccumulator like present-day A. thaliana via a secondary accumulator such as B. juncea. The physiological differences between nonaccumulators and secondary accumulators in Se accumulation and tolerance are mainly quantitative, and could be explained via higher expression levels of genes involved in sulfate/selenate uptake and assimilation, which could be the result of gene duplication events or mutations in regulatory sequences. Genetic variation in Se tolerance and accumulation has been shown to exist within the nonaccumulator species A. thaliana (Zhang et al., 2006a) as well as many crop species (as reviewed by White, 2016). Selection pressures during the evolution of secondary Se accumulation may include physiological and ecological benefits, as discussed in the previous section III. The maximum Se concentrations found to occur in secondary accumulators in the field (close to 1000 mg kg\(^{-1}\) DW) are also the concentrations at which tolerance breaks down in the secondary accumulator B. juncea (Prins et al., 2011) and may represent the secondary accumulator physiological tolerance ceiling. Hyperaccumulator species may have broken through this ceiling by evolving novel physiological mechanisms that enable them to tolerate extreme tissue Se concentrations, such as methylation of SeCys and specific sequestration of methyl-SeCys in epidermal structures; at the same time they have evolved a way to specifically sequester Se over S (selenate-specific transport?), and extremely high levels of sulfate/selenate assimilation (Freeman et al., 2010; Cappa et al., 2014). Characterization of Se tolerance and (hyper)accumulation within the various Stanleya taxa has indicated that true hyperaccumulation is
restricted to the *S. pinnata* clade, including var. *pinnata*, var. *bipinnata* and var. *integrifolia*. Two other varieties, *S. pinnata* var. *inyoensis* and var. *texana*, do not show the trait (Cappa et al., 2015). Better resolution of the *S. pinnata* clade is needed to assess how many times Se hyperaccumulation was gained and lost. Se hypertolerance and the capacity to store Se in epidermal cells have also been found in taxa outside the *S. pinnata* clade, and may have preceeded hyperaccumulation during evolution (Cappa et al., 2015).

**IV. Ecological aspects of plant Se (hyper)accumulation**

Ecological studies on plant–herbivore, plant–pollinator, plant–plant and plant–microbe interactions of Se hyperaccumulators reveal an emergent pattern: the high Se concentrations in hyperaccumulators tend to have a negative effect on Se-sensitive ecological partners, in which they cause toxicity, but allow the presence of or even facilitate Se-resistant ecological partners. The findings are summarized later for each type of ecological partner, as well as in Fig. 3.

1. **Plant–herbivore interactions**

Se was found to protect both hyperaccumulator and nonaccumulator species against a wide variety of herbivores with different feeding modes (Vickerman et al., 2002; Hanson et al., 2003, 2004; Freeman et al., 2007, 2009; Quinn et al., 2008, 2010a). The protection was found only when the plants were pretreated with Se, so presumably was a direct Se effect, and was attributable to both deterrence and toxicity. The deterrence may be attributable to smell, that is, volatile Se compounds, as was found for aphids, or to taste, as was found for caterpillars (Hanson et al., 2003, 2004). As Se protected both *S. pinnata* (which accumulates methyl-SeCys) and *B. juncea* (which accumulates selenate), both organic and
inorganic plant Se forms are protective. Tissue Se threshold concentrations that were protective depended on the herbivore species but in some cases were > 10 mg kg$^{-1}$ DW (Hanson et al., 2004). Based on these findings, it can be hypothesized that in nature plants with high Se concentrations will probably be avoided by most generalist herbivores and have a negative effect on them when eaten. In agreement with this hypothesis, high-Se plants were found to contain a lower invertebrate load in the field, and exhibited less herbivory damage (Galeas et al., 2008; El Mehdawi et al., 2015b).

While Se accumulation in plants apparently protects them from a wide variety of herbivores, hyperaccumulators are subject to herbivory in their natural habitat. There have been multiple reports of Se-resistant invertebrate herbivores that feed on hyperaccumulators without ill effects (Freeman et al., 2006a, 2012; Valdez et al., 2012). Thus, if Se can be considered an elemental defense, some herbivores have apparently overcome this defense, as has been found for other types of plant herbivore defenses (Boyd, 2010). Some of the herbivores found feeding on Se hyperaccumulators were resistant to the associated high Se ingestion because they could tolerate high tissue Se concentrations (Freeman et al., 2006a). Other herbivores found in seeds of Se hyperaccumulator species *S. pinnata* and *A. bisulcatus* appear to exclude Se in their gut, preventing it from entering their tissues and instead excreting it in the frass (Freeman et al., 2012). Such Se-resistant herbivores obviously benefit from their association with Se hyperaccumulators, being able to exclusively feed on this food source. Some may even be specialists, feeding exclusively on hyperaccumulators. Their extreme Se resistance may be the result of coevolution with their host. It will be interesting to further investigate this interaction. Interesting questions to explore are also whether Se-tolerant herbivores benefit from the ingested Se, either physiologically or via protection from predators and parasites. Furthermore, it is interesting to know whether Se-tolerant herbivores form a portal for Se into the food chain. This may be the case, as was found in a study by Vickerman & Trumble (2003) where Se-enriched *Spodoptera exigua* larvae negatively affected predator *Podisus maculiventris* via biotransfer of Se.

2. Plant–pollinator interactions

All organs of Se hyperaccumulators contain hyperaccumulator Se concentrations (> 0.1% of DW), but the highest concentrations are found in the flowers and seeds (Quinn et al., 2011). As high plant Se concentrations deter insect herbivores, as described in the previous section (plant–herbivore interactions), they could be hypothesized also to deter insect pollinators, thus causing reduced plant fitness. However, this was found to not be the case: the Se concentrations in the flowers did not affect visitation by potential pollinators (honey bees and native bumble bees) and the bees were found to incorporate Se into their tissues and to carry around collected Se-rich pollen in their pollen baskets (Quinn et al., 2011). These findings deserve further study: is there different Se resistance in the native and introduced pollinators or different foraging behavior, and what effect does ingestion of plant Se have on pollinator health? Studies by Hladun et al. (2012) have shown that there may indeed be a negative effect of Se ingestion by honey bees, depending on the form of Se. The bees did not avoid Se-containing food resources, which suggests that Se ingestion by honey bees is likely to happen in fields with Se-rich plants, in agreement with the field observations by Quinn et al. (2011).

3. Plant–plant interactions

The soil under the canopy of Se hyperaccumulators in the field in naturally Se-rich areas was found to contain c. 10-fold higher Se concentrations than soil at the same site further away (> 4 m) from hyperaccumulators (El Mehdawi et al., 2011a). This raises the question: do Se hyperaccumulators phytoenrich their surrounding soil with Se, via litter or root Se deposition, or was this soil already Se-rich and therefore favored the establishment of Se hyperaccumulators? Phytoenrichment is quite feasible, as Se hyperaccumulators are perennial forbs with extensive, deep root systems that scavenge large soil volumes, accumulate the Se in organic form and deposit high-Se leaf litter every fall, which loses most of its Se within 6 months, leading to elevated soil Se concentrations underneath the litter (Quinn et al., 2010b). Moreover, roots of Se hyperaccumulators were found to exude Se in organic form, and soil collected under the canopy of Se hyperaccumulators was found to contain a high fraction of organic Se (El Mehdawi et al., 2015a). If, indeed, Se hyperaccumulators exude Se into their rhizosphere and affect their surrounding soil Se concentration and chemical speciation via root and litter Se deposition, this probably affects belowground ecological partners.

Several studies carried out to date indicate that plants surrounding Se hyperaccumulators are influenced by their proximity to these high-Se plants. In a first experiment, soil collected from around hyperaccumulator species *S. pinnata* and *A. bisulcatus* was found to inhibit germination and growth of *A. thaliana*, as compared with soil collected around nonaccumulator species *Helianthus pumilus* and *Medicago sativa* growing on the same seleniferous site (El Mehdawi et al., 2011a). The plants that did grow on the hyperaccumulator-associated soil had elevated tissue Se concentrations. Furthermore, the soil Se concentrations found in the hyperaccumulator-associated soil were sufficient to inhibit germination and growth when supplied to the *A. thaliana* seedlings in agar medium. This suggests that the elevated Se concentrations in soil around hyperaccumulators are phytoxic to Se-sensitive plant species, and supports the hypothesis that Se hyperaccumulation may function as a form of elemental allelopathy, thus offering an ecological benefit to the hyperaccumulators. If this is the case, the plant canopy cover would be hypothesized to be lower around hyperaccumulators, and this trend was indeed found (El Mehdawi et al., 2011a).

The plant species that were found growing naturally around Se hyperaccumulators in seleniferous areas showed no signs of toxicity but rather appeared to benefit from their proximity to hyperaccumulators: they were bigger and showed less herbivory and herbivore load, which was probably attributable to their elevated tissue Se concentrations (El Mehdawi et al., 2011b). Thus, while the high-Se soil associated with Se hyperaccumulator species is toxic to Se-sensitive plant species, Se-tolerant plant species occur under natural field settings that are actually facilitated by growing next to
Se hyperaccumulators because they can accumulate more Se, which protects them from herbivory.

4. Plant–microbe interactions

In a first experiment to test the effect of Se on plant-associated fungi, Se accumulation was shown to protect *B. juncea* (accumulator) plants from two Se-sensitive pathogenic fungi (Hanson et al., 2003); therefore, Se may also protect hyperaccumulators in the field from Se-sensitive pathogens. However, from field observations it is clear that there are also fungal pathogens that can thrive on *S. pinnata* leaves (E. A. H. Pilon-Smits, pers. obs.). In other studies, a range of endophytic and rhizosphere fungi were found to live in association with Se hyperaccumulators (Wangelin et al., 2011; Valdez et al., 2012; Lindblom et al., 2013). Some of these were extremely Se resistant and could produce red elemental Se from selenite when cultured on agar plates. They may do the same when growing inside their hosts, as stems and roots of Se hyperaccumulators *S. pinnata* and *A. bisulcatus* were found to contain up to 30% elemental Se using X-ray microprobe analysis, but only when growing in the field and not when grown from surface-sterilized seeds in the laboratory (Lindblom et al., 2013). Thus, plants with high tissue Se concentrations may exclude Se-sensitive fungal partners but offer a niche for Se-resistant fungi, and endophytic fungi may affect plant speciation.

Nonculture-dependent studies have suggested that hyperaccumulators appear to harbor as diverse an endophytic bacterial community as nonaccumulators growing on the same seleniferous site; subsequent isolation and characterization of endophytic bacteria from hyperaccumulators showed that many were extremely Se resistant and could produce elemental Se from selenite (Sura-de Jong et al., 2015). X-ray microprobe analysis of an *A. bisulcatus* nodule (containing Rhizobacteria) and an adjacent root showed a high elemental Se fraction in the nodule but not the root (Valdez et al., 2012). Thus, endophytic bacteria may also contribute to the high elemental Se fractions found in some hyperaccumulators in the field, as mentioned in the previous paragraph. Nodulation of *Astragalus* species by Rhizobia was not negatively affected by their high Se concentrations, indicating that there is no cost of Se hyperaccumulation in terms of N acquisition (Alford et al., 2012, 2014). Rather, nodulation favored Se accumulation in the hyperaccumulator, and was associated with a c. 10-fold increased concentration of \( \gamma \)-glutamyl-methylSeCys (Alford et al., 2014). Thus, the nitrogen fixation capability of the rhizobial partner may be an important driver for the formation of organic selenocompounds in *Astragalus* and, consequently, Se hyperaccumulation. In another study, also investigating the effect of plant Se on microbial activity, high-Se litter was found to decompose faster than low-Se litter in a seleniferous area, and to harbor more cultivable microbes (Quinn et al., 2010b). In summary, so far there is no evidence of any negative effect of high plant Se concentrations on bacterial ecological partners, whether on or inside a living plant or during the process of plant litter decomposition. Some microbes may benefit from their ability to utilize organic selenocompounds, as they provide them with the essential elements carbon, nitrogen and Se.

5. Integrated ecological effects of plant Se accumulation

If, as appears to be the case, the high Se concentrations in and around hyperaccumulators have a negative effect on Se-sensitive ecological partners, but facilitate Se-resistant ecological partners, this may affect species composition in their local ecosystem. Hyperaccumulators may select against Se-sensitive plant, fungal and herbivore species and facilitate Se-resistant species. Hyperaccumulators may also affect Se cycling, directly or indirectly. By redistributing soil Se and transforming it from inorganic to more bioavailable organic forms, they may form an important portal for Se into the local food web, via Se-tolerant herbivores, detritivores and pollinators. This will be further amplified by continuous selective advantage for species at different trophic levels that can tolerate high tissue Se concentrations. Through these mechanisms, Se hyperaccumulators may have a relatively large effect on their local ecosystem, relative to their low abundance. This will be an interesting hypothesis to address in future research.

V. Future prospects

Plant Se research has many facets, and continues to reveal new insights at different levels, from the molecular level to global cycling. Questions that remain to be addressed regarding the basic science of plant Se metabolism include: (1) is Se really not essential for higher plants, including hyperaccumulators? (2) How does Se exert its beneficial effects on plants, and why are these effects so pronounced in hyperaccumulators? (3) How does Se interact with other beneficial plant compounds (nutraceuticals), and how can Se accumulation in plants be optimized for overall nutritional value? (4) Which are the molecular mechanisms that underlie hyperaccumulation; in particular, is there an Se-specific transporter, and a hyperaccumulation master switch? (5) How did hyperaccumulation evolve, and what are the evolutionary benefits and constraints? (6) How important are plants, including algae and hyperaccumulators, for Se cycling at the global and ecosystem levels? (7) To what extent do Se hyperaccumulators affect Se distribution and shape species composition in their local ecosystem? The advent of next-generation sequencing, including sequencing of genomes and transcriptomes of Se hyperaccumulators, should facilitate future studies on molecular mechanisms of Se hyperaccumulation and the evolution of hyperaccumulation in different clades, and may help to answer some of these ongoing questions. Better insight into fundamental Se-related plant processes is not only of intrinsic interest, but is relevant for applications of Se in medicine, industry and agriculture.

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