Selenium Metabolism in Plants

Elizabeth A.H. Pilon-Smits and Colin F. Quinn

Abstract Selenium (Se) is an essential nutrient for many organisms, but also toxic at higher levels. While certain algae require Se to make selenoproteins, no such requirement has been shown for higher plants. Still, plants readily take up and assimilate Se using sulfur (S) transporters and biochemical pathways, and can also volatilize methylated Se. Some plants can even hyperaccumulate Se to levels around 1% of plant dry weight, in the form of methyl-selenocysteine, probably as a defense mechanism. Plants may be used both to provide dietary Se in areas of Se deficiency, and to clean up Se pollution from seleniferous areas. These applications benefit from better insight into the genetic and biochemical mechanisms that control plant Se tolerance and accumulation. Here we give a review of plant Se metabolism, and present new insights into plant Se tolerance and hyperaccumulation mechanisms. Moreover, we summarize research on the ecological aspects of plant Se accumulation.

1 Introduction

The element selenium (Se) is chemically similar to sulfur (S), and as a result, plants and other organisms readily take up and metabolize Se via S transporters and pathways. Since replacement of S by Se in proteins and other S compounds disrupts the function of these molecules, Se is toxic at elevated levels to most organisms. For instance, a diet containing 1 mg/kg DW Se may lead to chronic Se poisoning in humans and animals, and one-time ingestion of plant material containing 1,000 mg/kg DW Se can lead to acute Se poisoning and death (Draize and Beath 1935; Rosenfeld and Beath 1964; Wilber 1980). Both chronic and acute Se poisoning are serious

E.A.H. Pilon-Smits (✉) and C.F. Quinn
Biology Department, Colorado State University, Anatomy/Zoology Building, Fort Collins, CO 80523, USA
e-mail: epsmits@lamar.colostate.edu; cquinn@lamar.colostate.edu

R. Hell and R.-R. Mendel (eds.), *Cell Biology of Metals and Nutrients*,
Plant Cell Monographs 17,
DOI 10.1007/978-3-642-10613-2_10,

problems in seleniferous areas such as in the Western USA, where Se is naturally present in soils derived from Cretaceous shale rock (Ohlendorf et al. 1986; Harris 1991; Kabata-Pendias 1998; Terry et al. 2000). On the other hand, Se is an essential trace element for many organisms including mammals, many bacteria, and certain green algae (Stadtman 1990, 1996; Fu et al. 2002). These organisms contain the so-called selenoproteins that contain selenocysteine (SeCys) in their active site. Interestingly, this SeCys is encoded by an opal stopcodon, which when in the right mRNA context, encodes SeCys instead. Se-requiring organisms can also contain seleno-tRNAs (Mihara and Esaki 2002). Selenoproteins invariably have antioxidant functions, including the scavenging of reactive oxygen species. In relation to this, Se deficiency is associated with an elevated probability of developing cancers or viral infections, as well as male infertility (Ellis et al. 2004; Diwadkar-Navsariwala et al. 2006; White and Broadley 2009). Extreme Se deficiency can lead to a type of heart disease termed white muscle disease in livestock and Keshan disease in people, after a province in China where this disease is common (Whanger 1989). The difference between the amount of Se required as a nutrient and the amount that is toxic is small; as a consequence both Se deficiency and toxicity are common problems worldwide (Terry et al. 2000). For higher plants, Se is known to be a beneficial nutrient but it has not been shown to be essential (Hartikainen 2005; Lyons et al. 2009; Pilon-Smits et al. 2009). Plant homologues of genes encoding selenoproteins in other organisms, such as glutathione peroxidase (GPX), were shown to encode a cysteine (Cys) instead of SeCys in the active site (Novoselov et al. 2002). Based on these *in silico* analyses it has been hypothesized that essential Se metabolism is a primitive trait that has been lost in evolution in higher plants and in other groups that do not require Se. It cannot be excluded, however, that some plants can post translationally convert an amino acid (e.g. serine) into selenocysteine, and thus produce selenoproteins differently. In this context it is interesting to note that Se treatment has been reported to enhance glutathione peroxidase activity in plants, and to reduce lipid peroxidation (Cartes et al. 2005; Djanaguiraman et al. 2005; Hartikainen 2005). Moreover, plants may have seleno-tRNAs; this has not been investigated.

While higher plants do not appear to require Se, they readily take it up from their environment and incorporate it into organic compounds using S assimilation enzymes, as depicted in Fig. 1. and, presented in more detail below. In short, inorganic selenate is reduced and assimilated into organic Se. The first organic form of Se produced is SeCys. This amino acid can be nonspecifically incorporated into proteins instead of Cys, leading to toxicity. An alternative fate of SeCys is to be converted to selenomethionine (SeMet), which also can be misincorporated into proteins, with less harmful effects. SeMet can also be converted to volatile dimethylselenide (DMSe), offering a release valve for excess Se from the plant (Lewis et al. 1966). SeCys can also be converted in plants to elemental Se and alanine (Pilon et al. 2003). Elemental Se is relatively innocuous; many bacteria use a similar Se detoxification mechanism. Furthermore, SeCys can be methylated, to form methyl-SeCys. This form of Se can safely be accumulated since it is not incorporated into proteins (Neuhierl et al. 1999). Methyl-SeCys can also act as

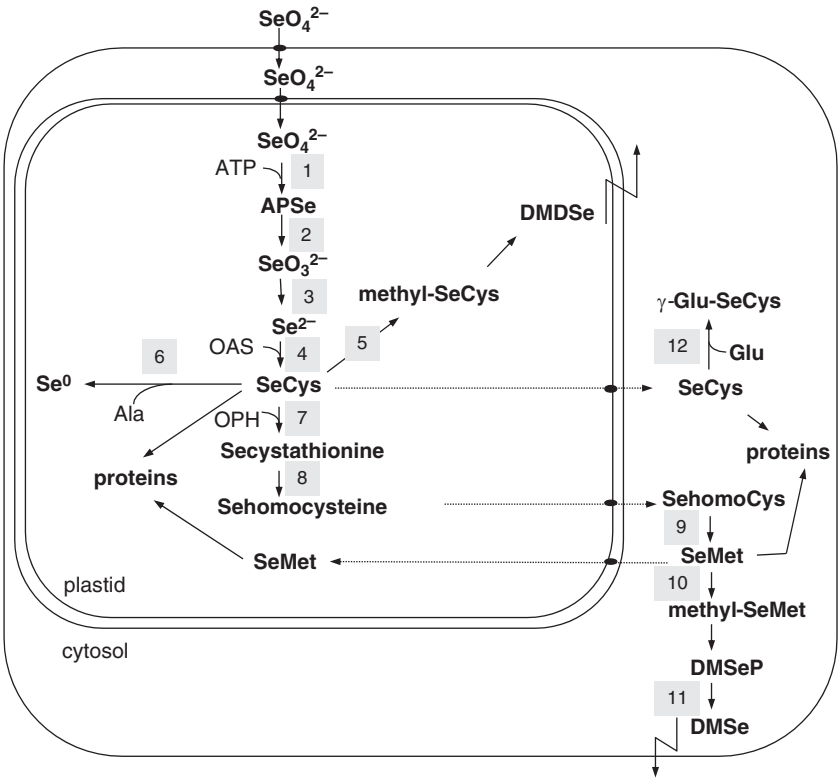

Fig. 1 Schematic overview of Se metabolism in plants. *APSe* adenosine phospho selenate, *SAT* serine acetyl transferase, *OAS O*-acetylserine, *OPH O*-phosphohomoserine, *SeCys* selenocysteine, *SeMet* selenomethionine, *DMSeP* dimethylselenoproprionate, *DMSe* dimethylselenide, *DMDSe* dimethyldiselenide. Numbers denote known enzymes. (1) ATP sulfurylase, (2) adenosine phosphosulfate reductase, (3) sulfite reductase (or glutathione), (4) OAS thiol lyase, (5) SeCys methyltransferase, (6) SeCys lyase, (7) cysthathionine-γ-synthase, (8) cysthathionine-β-lyase, (9) methionine synthase, (10) methionine methyltransferase, (11) DMSP lyase, (12) γ-glutamylcysteine synthetase

a precursor for the production of another form of volatile Se, dimethyldiselenide (DMDSe) (Terry et al. 2000; Sors et al. 2005).

In general, plants that have a tendency to accumulate high levels of S compounds, such as members of the Brassica genus (mustards and cabbages), also are good Se accumulators. Such S-loving species that accumulate Se to fairly high levels (0.1% of dry weight (DW), or 1,000 mg Se kg^{-1} DW) when supplied with adequate external Se levels are called accumulator species. Accumulator species likely do not have any Se-specific pathways but take up and metabolize Se and S indiscriminately, simply at elevated rates compared to nonaccumulators. Another category of plant species are the Se hyperaccumulators. These plants, from the families Brassicaceae, Fabaceae, and Asteraceae, are only found on seleniferous

soils and typically accumulate Se to levels 100-fold higher than the surrounding vegetation in the field (Beath et al. 1939a, b). Se hyperaccumulators preferentially take up Se over S, and hyperaccumulate Se up to1% of DW, or 10,000 mg Se kg^{-1} DW from soil containing as little as 2–10 mg Se kg^{-1} without suffering toxicity (Neuhierl and Böck 1996; Neuhierl et al. 1999; Persans and Salt 2000; Ellis et al. 2004; LeDuc et al. 2004). There is evidence that Se hyperaccumulators can distinguish between S and Se (White et al. 2007) and have Se-specific metabolism, as discussed in more detail below. It has been suggested that Se is essential for hyperaccumulators, since hyperaccumulators grow significantly better in the presence of Se. However, to date there is no proof that these plants require Se to complete their life cycle. The positive growth response of hyperaccumulators to Se may also be due to alleviation of phosphorus toxicity, since it was much less pronounced when plants were grown at lower phosphorus levels (Broyer et al. 1972). Below, an overview is given of Se metabolism in plants, both in nonhyperaccumulators and hyperaccumulators.

2 Metabolism of Se

2.1 From Selenate to Selenocysteine

Selenate is the predominant form of bioavailable Se in oxic soils and selenite is more abundant in anoxic wetland conditions. Both forms are readily taken up by plants. Selenate is taken up and distributed by means of sulfate-proton cotransporters (Smith et al. 1995). All of the sulfate transporters in plants likely can transport selenate as well (Leustek 1996; Yoshimoto et al. 2002, 2003; Hawkesford 2003; Maruyama-Nakashita et al. 2004). Selenate assimilation takes place predominantly in the leaf chloroplasts (Pilon-Smits et al. 1999). The reduction of selenate to selenite appears to be a rate-limiting step in the Se assimilation pathway, since most plants supplied with selenate accumulate predominantly selenate, while plants supplied with selenite accumulate organic Se (de Souza et al. 1998). The conversion of selenate to selenite involves the consecutive action of two enzymes (Fig. 1). ATP sulfurylase (APS) couples selenate to ATP, forming adenosine phosphoelenate (APSe) (Wilson and Bandurski 1958). This is subsequently reduced to selenite by APS reductase (APR). There are isozymes for APS and APR in both chloroplast and cytosol, but most of the selenate reduction likely takes place in the chloroplast. The further reduction of selenite to selenide may happen exclusively in the chloroplast if it is mediated by sulfite reductase, in analogy with sulfite reduction. However, it has also been suggested that nonenzymatic reduction by reduced glutathione (GSH) may play a significant role in selenite reduction (Anderson 1993; Terry et al. 2000). Selenide can subsequently be coupled to *O*-acetylserine (OAS) to form SeCys, by means of OAS thiol lyase (also called cysteine synthase). This enzyme activity is found in cytosol, chloroplasts, and mitochondria. OAS is synthesized by the

enzyme serine acetyl transferase, and functions as a signal molecule that upregulates the activity of sulfate transporters and sulfate assimilation enzymes.

2.2 From Selenocysteine to Other Selenocompounds

SeCys to SeMet and DMSe – SeCys can be converted to SeMet via the action of three enzymes (Fig. 1). The first, cystathionine-γ-synthase (CγS), couples SeCys to *O*-phosphohomoserine to form Se-cystathionine. The second enzyme, cystathionine-β-lyase, converts Se-cystathionine into Se-homocysteine. These first two enzymes are thought to be chloroplastic. The next step, however, occurs in the cytosol. Se-homocysteine is converted to SeMet via the action of Met synthase. SeMet has multiple possible fates, one of which is to be methylated to methyl-SeMet via the enzyme methionine methyltransferase. Methyl-SeMet can be further metabolized to volatile DMSe, which is cleaved off of the intermediate, dimethyl-selenopropionate (DMSeP), by DMSeP lyase.

SeCys to Se(0) – SeCys can be converted to elemental Se (Se(0)), via the action of a selenocysteine lyase (SL). NifS-like enzymes with SL activity have been found in both chloroplasts and mitochondria (Pilon et al. 2003). In organisms that require Se, SL enzymes provide elemental Se for selenoproteins and Se-tRNAs (Mihara and Esaki 2002). Overexpression of the chloroplastic plant SL (called cpNifS) was shown to reduce incorporation of Se into proteins as well as to enhance Se accumulation (Van Hoewyk et al. 2005). Whether this SL activity has any function *in vivo* is questionable. The main function of the NifS-like enzymes in plants is probably to act as Cys desulfurases in S metabolism, providing elemental S for iron–sulfur cluster formation (Van Hoewyk et al. 2007).

SeCys to Methyl-SeCys and DMDSe – SeCys can be methylated to form methyl-SeCys, via the action of SeCys methyltransferase (SMT). SMT enzyme activity is particularly pronounced in hyperaccumulators, and as a result, these species accumulate Se predominantly in the form of methyl-SeCys when supplied with selenate, while most other species accumulate selenate (de Souza et al. 1998; Freeman et al. 2006b). Since Methyl-SeCys does not enter proteins, it can be safely accumulated, explaining in part the tolerance of hyperaccumulators to Se. Recently, a Brassica species (*B. oleracea*, broccoli) was also shown to have an SMT enzyme, which was only expressed in the presence of Se (Lyi et al. 2005). Methyl-SeCys can be further converted to volatile DMDSe, the predominant volatile form of Se produced by Se hyperaccumulators (Terry et al. 2000; Kubachka et al. 2007). Hyperaccumulators also have been found to couple glutamate to methyl-SeCys, to form γ-glutamyl-methyl-SeCys, a major storage form of Se in hyperaccumulator seeds (Freeman et al. 2007; Kubachka et al. 2007). The enzyme mediation of this reaction is likely to be γ-glutamylcysteine synthetase (ECS). In S metabolism, this same enzyme functions in glutathione production (Glu–Cys–Gly). Reduced glutathione (GSH) has many redox functions in cells, and also is a negative regulator that downregulates S uptake and assimilation.

3 Genetic Engineering of Plant Se Metabolism

3.1 Results Obtained from Various Transgenic Approaches

As described above, all plants can take up inorganic selenate and selenite and assimilate them to SeCys and other organic selenocompounds, including volatile forms. Hyperaccumulators of Se may have additional metabolic pathways for Se, particularly methylation of SeCys and the conversion of methyl-SeCys to volatile DMDSe. To further enhance plant Se accumulation, tolerance, and volatilization, various transgenic approaches have been used.

One approach involved upregulation of key genes involved in S/Se assimilation and volatilization. First, overexpression of the first gene involved in selenate-to-selenite conversion, ATP sulfurylase (APS) in *Brassica juncea* (Indian mustard) resulted in enhanced selenate reduction, judged from the observation that the transgenic APS plants accumulated an organic form of Se when supplied with selenate, while wildtype (untransformed) controls accumulated selenate (Pilon-Smits et al. 1999). The APS transgenics accumulated two to threefold more Se than wild type, and 1.5-fold more S. The APS plants tolerated the accumulated Se better than wild type, perhaps because of the different form of Se accumulated. Se volatilization rate was not affected in the APS transgenics.

Second, overexpression in *B. juncea* of the first enzyme in the conversion of SeCys to SeMet, cystathionine-γ-synthase (CgS), resulted in two to threefold higher volatilization rates compared to untransformed plants (Van Huysen et al. 2003). Probably as a result of their enhanced volatilization, the CgS transgenics accumulated 40% less Se in their tissues than wild type. The CgS transgenics were also more Se tolerant than wildtype plants, probably due to their lower tissue Se levels.

Another genetic engineering approach to manipulate plant Se metabolism targeted SeCys, and particularly the prevention of the toxic process of its nonspecific incorporation into proteins. A mouse SL was expressed in *A. thaliana* and *B. juncea* (Pilon et al. 2003; Garifullina et al. 2003). This enzyme specifically breaks down SeCys into alanine and elemental Se. The SL transgenics showed reduced Se incorporation into proteins. When expressed in the cytosol of *A. thaliana*, the mouse SL enhanced plant Se tolerance, but when expressed in the chloroplast Se tolerance was reduced (Pilon et al. 2003). Perhaps the produced elemental Se interfered with iron–sulfur cluster formation in this compartment, which uses elemental S. All the transgenic SL plants showed enhanced Se accumulation, up to twofold compared to wildtype plants. Later, similar results were obtained when an *A. thaliana* homologue of the mouse SL (called CpNifS) was discovered and overexpressed: the CpNifS transgenics showed less Se incorporation in proteins, twofold enhanced Se accumulation, as well as enhanced Se tolerance (Van Hoewyk et al. 2005).

In another approach to prevent SeCys incorporation into proteins, SeCys methyltransferase (SMT) from the Se hyperaccumulator *A. bisulcatus* was overexpressed in *A. thaliana* and *B. juncea* (Ellis et al. 2004; LeDuc et al. 2004). The SMT transgenics showed enhanced Se accumulation, in the form of methyl-SeCys, as

well as enhanced Se tolerance. The expression of SMT also resulted in increased rates of Se volatilization, with more volatile Se produced in the form of DMDSe.

While the expression of SMT enhanced Se tolerance, accumulation, and volatilization, the effects were more pronounced when the plants were supplied with selenite as opposed to selenate. Thus, the conversion of selenate to selenite appeared to be a rate-limiting step for the production of SeCys. To overcome this rate-limitation, APS and SMT transgenics were crossed to create double-transgenic plants that overexpress both APS and SMT (APSxSMT plants). The APS x SMT double transgenics accumulated up to nine times higher Se levels than wild type (LeDuc et al. 2006). Most of the Se in the double transgenics was in the form of methyl-SeCys: the APSxSMT plants accumulated up to eightfold more methyl-SeCys than wild type and nearly twice as much as the SMT transgenics. Se tolerance was similar in the single and double transgenics.

3.2 Obtained Insight into Rate-controlling Steps and Se Detoxification Mechanisms

From the genetic engineering studies we can conclude that the sulfate assimilation and volatilization pathway is capable of selenate assimilation and volatilization as well. The enzyme APS appears to be rate-limiting for the assimilation of selenate to organic Se, while CgS is rate-limiting for DMSe volatilization. Enhanced APS expression also appears to trigger selenate uptake and Se and S accumulation, likely due to upregulation of sulfate transporter expression.

The results from the SL and CpNifS transgenics show that the specific breakdown of SeCys can reduce nonspecific incorporation of Se into proteins. As long as the elemental Se does not interfere with cellular processes, this enhances Se tolerance. As mentioned above, whether CpNifS in plants functions in Se tolerance in nature is unknown; it's most important function is likely in synthesis of iron–sulfur clusters (Van Hoewyk et al. 2007). Since overexpression of SL or CpNifS led to enhanced, Se accumulation it appears that introduction of this new sink for Se upregulated Se and S uptake. The results from the SMT transgenics show that SMT is a key enzyme for Se hyperaccumulation, conferring enhanced Se tolerance and accumulation when expressed in nonhyperaccumulators. However, for optimal Se assimilation and detoxification, APS needs to be overexpressed together with SMT. APS x SMT double transgenics combine the ability to reduce selenate to selenite and SeCys with the ability to methylate SeCys and thus to detoxify the increased pool of internal Se.

3.3 Testing the Potential of the Transgenics for Phytoremediation, and as Fortified Foods

As described above, several different transgenics have been obtained that showed enhanced Se tolerance, accumulation, and assimilation from inorganic to organic

Se, and volatilization. Se accumulation was up to ninefold higher and volatilization up to threefold faster, under laboratory conditions. These properties may be useful for cleanup of excess levels of Se in the environment (phytoremediation), and also as fortified foods to prevent Se deficiency. Accumulators of MetSeCys would be particularly useful for the latter purpose, since this form of Se is particularly anticarcinogenic (Unni et al. 2005). In a first step to assess the transgenics' potential for phytoremediation or as Se-fortified food, they were tested for their capacity to accumulate Se from naturally seleniferous soil and from Se-contaminated sediment.

When grown on naturally seleniferous soil in a greenhouse pot experiment, the APS transgenics accumulated Se to threefold higher levels than wildtype *B. juncea*, and the CgS transgenics contained 40% lower Se levels than wild type (Van Huysen et al. 2004). These results are in agreement with the laboratory results. Plant growth was the same for all plant types in this experiment. Subsequently, a field experiment was carried out on Se (selenate)-contaminated sediment in the San Joaquin Valley (CA, USA) by Gary Banuelos and coworkers (Bañuelos et al. 2005). The APS transgenics accumulated Se to fourfold higher levels than wildtype *B. juncea*, which is similar to the laboratory and greenhouse results. In a second field experiment on the same Se-polluted sediment, the cpSL and SMT transgenics showed twofold higher Se accumulation than wildtype *B. juncea*, also in agreement with earlier laboratory experiments (Bañuelos et al. 2007). In both field experiments, biomass was comparable for the different plant types. Thus, the results obtained from the different transgenics using naturally seleniferous or Se-contaminated soils in a greenhouse or field are similar to those obtained under controlled laboratory conditions. The various transgenics showed enhanced Se accumulation, volatilization and/or tolerance, all promising traits for use as Se-fortified foods or for phytoremediation.

4 New Insights into Plant Se Responses and Tolerance Mechanisms

4.1 Results Using the Model Nonaccumulator Species Arabidopsis thaliana

Comparative studies have been performed using relatively Se-tolerant *A. thaliana* accessions versus nontolerant accessions, with the aim to reveal new insight into the genes that control Se uptake, (hyper) accumulation, and volatilization. Several quantitative trait loci (QTL) were identified that cosegregated with the higher selenate tolerance in *A. thaliana* accession Columbia compared with accession Landsberg erecta, using a population of recombinant inbred lines (Zhang et al. 2006a). Several genes involved in S assimilation are located in one of the identified chromosomal regions, which may be responsible for the Se tolerance conferred by this QTL. Other genetic and biochemical studies using 3 and 19 *A. thaliana*

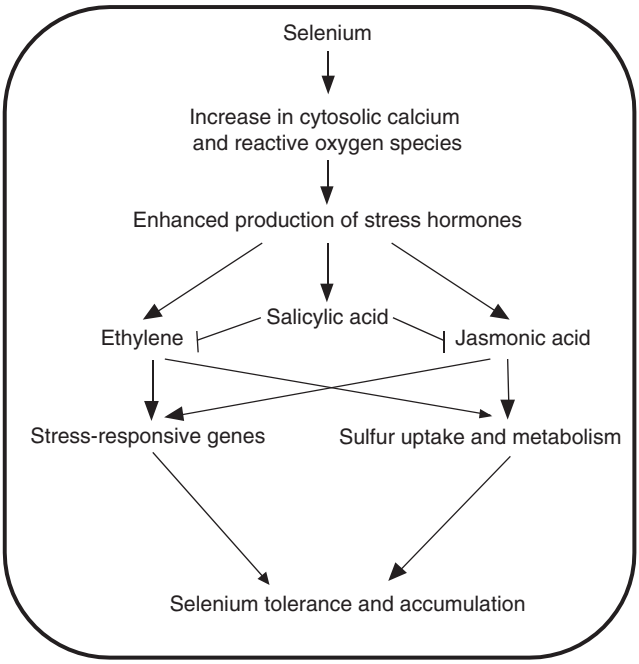

Fig. 2 Working model of Se responses contributing to Se tolerance in Arabidopsis (from data presented by Tamaoki et al. 2008)

accessions, respectively, have given further insight into Se tolerance and accumulation mechanisms in this species (Zhang et al. 2006b,c). Tolerance to selenate and selenite appear to be controlled at least in part by different loci, and tolerance and accumulation are not correlated. Genomic, genetic, and biochemical studies using Arabidopsis accessions differing in selenite or selenate tolerance revealed an important role for the plant hormones jasmonic acid (JA) and ethylene (Tamaoki et al. 2008; Van Hoewyk et al. 2008). Reactive oxygen species (ROS) may also have a signaling role, and the resistance mechanism appears to involve enhanced sulfate uptake and reduction, which may serve to prevent Se from replacing S in proteins and other S compounds. A working model for Se responses and tolerance is shown in Fig. 2.

4.2 Results Using Se Hyperaccumulators and Related Nonhyperaccumulators

In comparative studies using hyperaccumulator and nonhyperaccumulator species from the genus *Stanleya*, indications of similar Se tolerance mechanisms were found to those described above for Arabidopsis (Freeman, Tamaoki and Pilon-Smits,

unpublished results). In this genus, salicylic acid may play an additional role, besides JA and ethylene, and ROS levels were lower rather than higher in the Se-tolerant taxa. As mentioned above, selenate-supplied hyperaccumulators store Se predominantly as MeSeCys, while nonaccumulators such as Arabidopsis and accumulators such as *B. juncea* store mainly selenate, indicating differences in Se metabolic pathways. Se hyperaccumulator taxa also show interesting Se sequestration patterns that are not observed in nonhyperaccumulators. Around 90% of the accumulated Se is present as methyl-SeCys in specialized cells in the leaf epidermis or in leaf hairs (Freeman et al. 2006a). This may indicate the presence in hyperaccumulators of special transport mechanisms for selenocompounds into these specialized cell types. The observation that Se hyperaccumulators generally have much higher Se/S ratios compared to nonhyperaccumulators growing on the same soil (Feist and Parker 2001) also indicates the presence of specialized Se-specific transporters, perhaps exclusive selenate transporters that have evolved from sulfate transporters. Indeed, a study of seasonal fluctuations in Se and S levels in Se hyperaccumulators and related nonhyperaccumulators growing on the same field site indicated different fluxes for Se and S in hyperaccumulators, but not for nonaccumulators. Leaf Se concentration in hyperaccumulators peaked in early spring, while leaf S concentration peaked in summer. In nonaccumulators both Se and S levels peaked in summer (Galeas et al. 2007).

5 Ecological Aspects of Plant Se Accumulation

5.1 Contribution of Microbes to Se Uptake and Volatilization

The fluxes and metabolic conversions of Se through plants are summarized in Fig. 3. So far, studies on plant Se metabolism have generally been done using nonsterile plants. Since all plants live in symbiosis with a host of bacteria and fungi, and since these microbes can metabolize and volatilize Se as well, (Thompson-Eagle et al. 1989; de Souza and Terry 1997; Pankiewicz et al. 2006), an important question is: what role do plant-associated microbes play in plant Se accumulation and volatilization? Different groups of plant-associated microbes may affect Se uptake and volatilization in plants: rhizosphere microbes, living in the area that is under the influence of the plant root, endophytic microbes that live within plant tissues, as well as microbes that live on leaf surfaces. The microbes may be envisioned to help plants take up Se via different mechanisms, or to help them metabolize it and volatilize it.

There is convincing evidence that bacteria contribute to plant Se uptake and volatilization. In broccoli (*B. oleracea*) 95% of Se root volatilization was inhibited when roots were treated with the antibiotics chlortetracycline and penicillin (Zayed and Terry 1994). Similarly, Indian mustard (*B. juncea*) plants treated with the antibiotic ampicillin volatilized 30% less Se and accumulated 70% less Se than

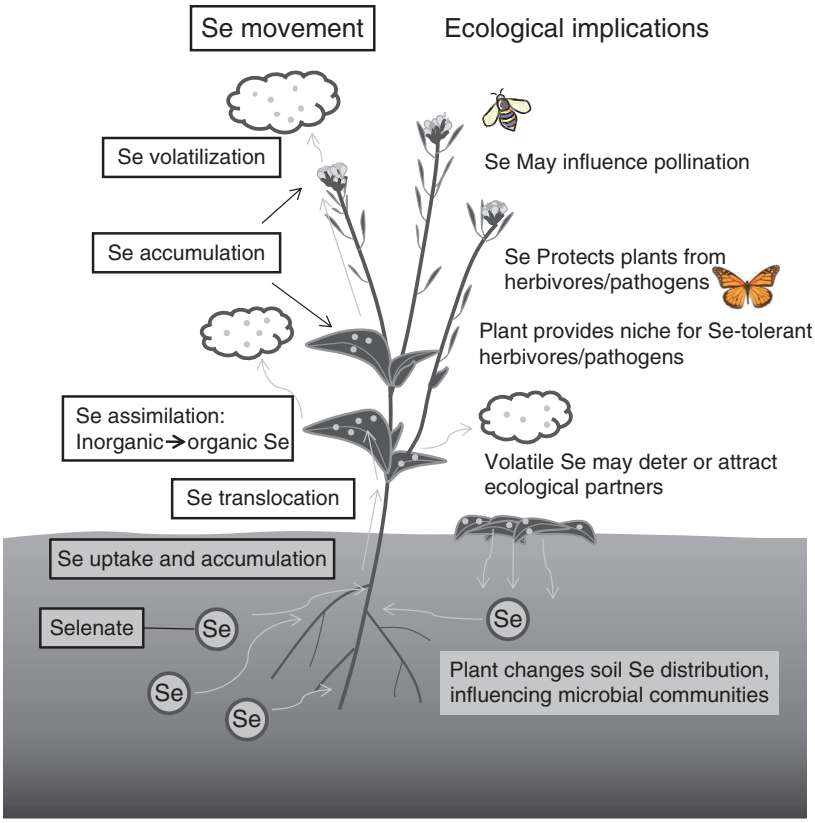

Fig. 3 Overview of the movement and metabolic conversion of Se by plants (*left side*) and their ecological implications (*right side*)

untreated plants. In addition, Indian mustard plants grown from surfaced-sterilized seeds that were subsequently inoculated with rhizospheric bacteria accumulated fivefold more Se and volatilized fourfold more Se than control plants from surface sterilized seeds that were not inoculated with bacteria. The mechanism for the stimulatory effect by the bacteria appeared to be both stimulation of root growth, and stimulation of S/Se uptake and assimilation. Plants inoculated with rhizospheric bacteria had an increased root surface area and the culture media contained ninefold higher serine levels than control plants. OAS is known to stimulate S uptake and assimilation (de Souza et al. 1999).

Less is known about a possible role for plant-associated fungi in plant Se uptake and volatilization. In one study, the nonaccumulator ryegrass accumulated less Se when treated with the mycorrhizal fungus *Glomus mosseae* compared to controls lacking in mycorrhizal fungus (Munier-Lamy et al. 2007). Also virtually nothing is known about the role endophytic microbes may play in Se uptake and volatilization. These will be interesting areas for further study.

5.2 *Effects of Plant Se on Ecological Partners*

The high Se levels in hyperaccumulators likely play an important role in the ecology of these plants, and even Se accumulated in crop plants may have ecological effects. These potential ecological implications of Se accumulation and volatilization by plants are depicted in Fig. 3. Most well known effects of plant Se hyperaccumulation on other species are the toxic effects of plant Se on livestock herbivores. Se poisoning due to ingestion of hyperaccumulator plants is responsible for losses of cattle, sheep and horses to the extent of hundreds of millions of US $ annually in the USA alone (Wilber 1980). In laboratory and field studies, Se accumulation was shown to protect plants from a wide variety of herbivores and pathogens, ranging from prairie dogs to a variety of arthropods and fungi (Hanson et al. 2003, 2004; Freeman et al. 2006a, 2007; Quinn et al. 2008). This protective effect of Se was both due to deterrence and toxicity. These studies lend support to the elemental defence hypothesis, which states that plants hyperaccumulate metals as protection against herbivory and pathogen attacks (Boyd and Martens 1993). In their natural habitat in the field, Se hyperaccumulating species harbored fewer arthropod species and individuals than comparable Se nonaccumulators (Galeas et al 2008).

Herbivores that ingest hyperaccumulator plant material readily convert the ingested methyl-SeCys to SeCys (Freeman et al. 2006b), which is toxic because of its inadvertent incorporation into proteins. Thus, Se hyperaccumulation is an effective plant defense mechanism against herbivory. Like all plant defense, over time, some herbivores will evolve tolerance. Indeed, a population of diamondback moth living in a seleniferous area was shown to have evolved Se tolerance (Freeman et al. 2006b). This population of diamondback moth was found feeding primarily on the Se hyperaccumulator *S. pinnata*. The mechanism for Se tolerance appears to be metabolic: the Se-tolerant moth accumulated Se in the ingested form, methyl-SeCys, which is not incorporated into proteins, while a control population of diamondback moth from a nonseleniferous habitat was Se-sensitive and converted the ingested methyl-SeCys to SeCys (Freeman et al. 2006b).

Another mechanism herbivores may utilize to minimize Se toxicity is to avoid Se-rich plant tissue. Se is not distributed evenly throughout Se hyperaccumulating plants, and Se levels fluctuate over the growing season. Leaf Se concentrations peak in early spring, and are much higher in young leaves and reproductive tissues than in older leaves (Galeas et al. 2007). Also, within the flowers of *S. pinnata*, the stamens and pistils have a higher Se concentration than the petals and sepals (Quinn et al unpublished results). Furthermore, hyperaccumulators preferentially allocate Se to the periphery of the leaf, unlike nonhyperaccumulators. In *S. pinnata*, Se is stored primarily in specialized cells in the epidermis, and in *A. bisulcatus* Se is stored primarily in leaf hairs (Freeman et al. 2006b). Therefore, it appears that hyperaccumulators preferentially allocate Se to their most valuable tissues, for protection from herbivores and pathogens. Sequestration in the epidermis may also contribute to Se tolerance. Depending on herbivore feeding mode,

Se hyperaccumulation may be more or less effective against different herbivores. In view of the particularly high Se levels in the flowers,Se may also play a role in pollination ecology. This will be an interesting area of further research

The elevated Se levels in and around hyperaccumulator plants likely also affects local microbial communities. Soil around Se hyperaccumulating plants has a ~tenfold higher Se concentration than the surrounding bulk soil, and there is evidence that rhizosphere and saprophytic fungi from seleniferous areas have evolved enhanced Se tolerance (Wangeline and Pilon-Smits, unpublished results; Quinn and Pilon-Smits, unpublished results).

6 Conclusions and Future Prospects

Building on the genomic and biochemical studies described above, follow-up research may reveal key genes that trigger the cascade of responses that together provide Se tolerance and accumulation in model plants and hyperaccumulators. Also, genes may be found that encode specific transporters of selenocompounds into and within hyperaccumulators. Such key genes will be the ultimate candidates for overexpression studies, with the potential of transferring the complete Se hyperaccumulator profile into high-biomass species.

Recent research has elucidated many important ecological interactions involving Se in plants. This research has helped identify important areas for future research. Particularly, more research is warranted on the role microbes play in plant Se uptake and volatilization, and the movement of Se through the food chain via Se hyperaccumulators or Se-fortified crop plants. The role of Se in below-ground ecological interactions with microbes and other organisms is also a fairly un-explored area. In addition to effects of Se on root–microbe interactions, Se may protect plants from root feeding herbivores, and selenocompounds released from hyperaccumulator roots may be toxic to surrounding vegetation. Similarly, the effects of Se on pollination ecology will be an interesting field of further study.

Better knowledge of the processes involved in plant metabolism of Se, the limiting factors involved, the contributions of ecological partners and the effects of Se on ecological partners are all useful for minimizing potential harmful effects of Se while benefiting from the positive effects of plant Se on animal and human health.

The capacity of plants to accumulate and volatilize Se will be very useful for the phytoremediation of Se-contaminated soils and waters (Bañuelos and Meek 1990; Hansen et al. 1998). When plant Se accumulation is managed well, this offers an efficient and cost-effective way to remove Se from the environment. Since plants are an effective source of dietary Se, Se-enriched plant material from phytoremediation or other sources can be considered fortified food. After being grown on Se-contaminated soil or being irrigated with Se-contaminated water, the Se-laden plant material may be used as a feed supplement for livestock, or as a biofuel. If successful, the potential of this strategy may be further enhanced

by the use of selected transgenic lines. Of course, any use of Se-accumulating wildtype or transgenic plants will need to be accompanied by careful risk assessment, to avoid escape of transgenes and any adverse ecological effects of the accumulated Se.

Acknowledgements The writing of this manuscript was supported by National Science Foundation grant # IOS-0817748 to EAHPS. We thank Wiebke Tapken for creative design of figures. We thank all researchers who contributed to the results described here and apologize to those whose work we could not cover due to space limitation.

References

Anderson JW (1993) Selenium interactions in sulfur metabolism. In: De Kok LJ (ed) Sulfur nutrition and assimilation in higher plants – regulatory, agricultural and environmental aspects. SPB Academic, The Netherland, pp 49–60

Bañuelos GS, Meek DW (1990) Accumulation of selenium in plants grown on selenium-treated soil. J Environ Qual 19:772–777

Bañuelos G, Terry N, LeDuc DL, Pilon-Smits EAH, Mackey B (2005) Field trial of transgenic Indian mustard plants shows enhanced phytoremediation of selenium contaminated sediment. Environ Sci Technol 39:1771–1777

Bañuelos G, LeDuc DL, Pilon-Smits EAH, Tagmount A, Terry N (2007) Transgenic Indian mustard overexpressing selenocysteine lyase, selenocysteine methyltransferase, or methionine methyltransferase exhibit enhanced potential for selenium phytoremediation under field conditions. Environ Sci Technol 41:599–605

Beath OA, Gilbert CS, Eppson HF (1939a) The use of indicator plants in locating seleniferous areas in Western United States. I. General. Am J Bot 26:257–269

Beath OA, Gilbert CS, Eppson HF (1939b) The use of indicator plants in locating seleniferous areas in Western United States. II. Correlation studies by states. Amer J Bot 26:296–315

Boyd RS, Martens SN (1993) The raison d'etre for metal hyperaccumulation by plants. In: Baker AJM, Proctor J, Reeves RD (eds) The vegetation of ultramafic (serpentine) soils. Intercept, Andover, UK, 279–289

Broyer TC, Huston RP, Johnson CM (1972) Selenium and nutrition of Astragalus. 1. Effects of selenite or selenate supply on growth and selenium content. Plant Soil 36:635–649

Cartes P, Gianfreda L, Mora ML (2005) Uptake of selenium and its antioxidant activity in ryegrass when applied as selenate and selenite forms. Plant Soil 276:359–367

de Souza MP, Terry N (1997) Selenium volatilization by rhizosphere bacteria. Abstr Gen Meet Am Soc Microbiol 97:499

de Souza MP, Pilon-Smits EAH, Lytle CM, Hwang S, Tai JC, Honma TSU, Yeh L, Terry N (1998) Rate-limiting steps in selenium volatilization by *Brassica juncea*. Plant Physiol 117:1487–1494

de Souza MP, Chu D, Zhao M, Zayed AM, Ruzin SE, Schichnes D, Terry N (1999) Rhizosphere bacteria enhance selenium accumulation and volatilization by Indian mustard. Plant Physiol 119:565–574

Djanaguiraman M, Durga Devi D, Shanker AK, Sheeba JA, Bangarusamy U (2005) Selenium – an antioxidative protectant in soybean during selenscence. Plant Soil 272:77–86

Diwadkar-Navsariwala V, Prins GS, Swanson SM, Birch LA, Ray VH, Hedayat S, Lantvit DL, Diamond AM (2006) Selenoprotein deficiency accelerates prostate carcinogenesis in a transgenic model. Proc Natl Acad Sci USA 103:8179–8184

Draize JH, Beath OA (1935) Observation on the pathology of "blind staggers" and "alkali disease". Am Vet Med Assoc J 86:53–763

Ellis DR, Sors TG, Brunk DG, Albrecht C, Orser C, Lahner B, Wood KV, Harris HH, Pickering IJ, Salt DE (2004) Production of Se-methylselenocysteine in transgenic plants expressing selenocysteine methyltransferase. BMC Plant Biol 4:1–11

Feist LJ, Parker DR (2001) Ecotypic variation in selenium accumulation among populations of *Stanleya pinnata*. New Phytol 149:61–69

Freeman JL, Quinn CF, Marcus MA, Fakra S, Pilon-Smits EAH (2006a) Selenium tolerant diamondback moth disarms hyperaccumulator plant defense. Current Biol 16:2181–2192

Freeman JL, Zhang LH, Marcus MA, Fakra S, McGrath SP, Pilon-Smits EAH (2006b) Spatial imaging, speciation and quantification of selenium in the hyperaccumulator plants *Astragalus bisulcatus* and *Stanleya pinnata*. Plant Physiol 142:124–134

Freeman JL, Lindblom SD, Quinn CF, Fakra S, Marcus MA, Pilon-Smits EAH (2007) Selenium accumulation protects plants from herbivory by orthoptera due to toxicity and deterrence. New Phytol 175:490–500

Fu L-H, Wang X-F, Eyal Y, She Y-M, Donald LJ, Standing KG, Ben-Hayyim G (2002) A selenoprotein in the plant kingdom: Mass spectrometry confirms that an opal codon (UGA) encodes selenocysteine in *Chlamydomonas reinhardtii* glutathione peroxidase. J. Biol Chem 277:25983–25991

Galeas ML, Zhang LH, Freeman JL, Wegner M, Pilon-Smits EAH (2007) Seasonal fluctuations of selenium and sulfur accumulation in selenium hyperaccumulators and related non-accumulators. New Phytol 173:517–525

Galeas ML, Klamper EM, Bennett LE, Freeman JL, Kondratieff BC, Pilon-Smits EAH (2008) Selenium hyperaccumulation affects plant arthropod load in the field. New Phytol 177:715–724

Garifullina GF, Owen JD, Lindblom S-D, Tufan H, Pilon M, Pilon-Smits EAH (2003) Expression of a mouse selenocysteine lyase in *Brassica juncea* chloroplasts affects selenium tolerance and accumulation. Physiol Plant 118:538–544

Hansen D, Duda PJ, Zayed A, Terry N (1998) Selenium removal by constructed wetlands: role of biological volatilization. Environ Sci Technol 32:591–597

Hanson BR, Garifullina GF, Lindblom SD, Wangeline A, Ackley A, Kramer K, Norton AP, Lawrence CB, Pilon-Smits EAH (2003) Selenium accumulation protects *Brassica juncea* from invertebrate herbivory and fungal infection. New Phytol 159:461–469

Hanson BR, Lindblom SD, Loeffler ML, Pilon-Smits EAH (2004) Selenium protects plants from phloem-feeding aphids due to both deterrence and toxicity. New Phytol 162:655–662

Harris T (1991) Death in the Marsh. Island Press, Washington, DC

Hartikainen H (2005) Biogeochemistry of selenium and its impact on food chain quality and human health. J Trace Elem Med Biol 18:309–318

Hawkesford MJ (2003) Transporter gene families in plants: the sulphate transporter gene family – redundancy or specialization? Physiol Plant 117:155–163

Kabata-Pendias A (1998) Geochemistry of selenium. J Environ Pathol Toxicol Oncol 17:173–177

Kubachka KM, Meija J, LeDuc DL, Terry N, Caruso JA (2007) Environ Sci Technol 41:1863–1869

LeDuc DL, Tarun AS, Montes-Bayon M, Meija J, Malit MF, Wu CP, AbdelSamie M, Chiang C-Y, Tagmount A, deSouza MP, Neuhierl B, Bock A, Caruso JA, Terry N (2004) Overexpression of selenocysteine methyltransferase in *Arabidopsis* and indian mustard increases selenium tolerance and accumulation. Plant Physiol 135:377–383

LeDuc DL, AbdelSamie M, Montes-Bayón M, Wu CP, Reisinger SJ, Terry N (2006) Over-expressing both ATP sulfurylase and selenocysteine methyltransferase enhances selenium phytoremediation traits in Indian mustard. Environ Pollut 144:70–76

Leustek T (1996) Molecular genetics of sulfate assimilation in plants. Physiol Plant 97:411–419

Lewis BG, Johnson CM, Delwiche CC (1966) Release of volatile selenium compounds by plants: collection procedures and preliminary observations. J Agric Food Chem 14:638–640

Lyi SM, Heller LI, Rutzke M, Welch RM, Kochian LV, Li L (2005) Molecular and biochemical characterization of the selenocysteine Se-methyltransferase gene and Se-methylselenocysteine synthesis in broccoli. Plant Physiol 138:409–420

Lyons GH, Genc Y, Soole K, Stangoulis JCR, Liu F, Graham RD (2009) Selenium increases seed production in *Brassica*. Plant Soil 318:73–80

Maruyama-Nakashita A, Nakamura Y, Yamaya T, Takahashi H (2004) A novel regulatory pathway of sulfate uptake in *Arabidopsis* roots: implication of CRE1/WOL/AHK4-mediated cytokinin-dependent regulation. Plant J 38:779–789

Mihara H, Esaki N (2002) Bacterial cysteine desulfurases: their function and mechanisms. Appl Microbiol Biotechnol 60:12–23

Munier-Lamy C, Deneux-Mustin S, Mustin C, Merlet D, Berthelin J, Leyval C (2007) Selenium bioavailability and uptake as affected by four different plants in a loamy clay soil with particular attention to mycorrhizae inoculated ryegrass. J Environ Radioact 97:148–158

Neuhierl B, Böck A (1996) On the mechanism of selenium tolerance in selenium accumulating plants. Purification and characterization of a specific selenocysteine methyltransferase from cultured cells of *Astragalus bisulcatus*. Eur J Biochem 239:235–238

Neuhierl B, Thanbichler M, Lottspeich F, Böck A (1999) A family of S-methylmethionine dependent thiol/selenol methyltransferases. Role in selenium tolerance and evolutionary relation. J Biol Chem 274:5407–5414

Novoselov SV, Rao M, Onoshko NV, Zhi H, Kryukov GV, Xiang Y, Weeks DP, Hatfield DL, Gladyshev VN (2002) Selenoproteins and selenocysteine insertion system in the model plant system, *Chlamydomonas reinhardtii*. EMBO J 21:3681–3693

Ohlendorf HM, Hoffman DJ, Salki MK, Aldrich TW (1986) Embryonic mortality and abnormalities of aquatic birds: apparent impacts of selenium from irrigation drain water. Sci Total Environ 52:49–63

Pankiewicz U, Jamroz J, Schodziñski A (2006) Optimization of selenium accumulation in *Rhodotorula rubra* cells by treatment of culturing medium with pulse electric field. Int Agrophysics 20:147–152

Persans MW, Salt DE (2000) Possible molecular mechanisms involved in nickel, zinc and selenium hyperaccumulation in plants. Biotechnol Genet Eng Rev 17:389–413

Pilon M, Owen JD, Garifullina GF, Kurihara T, Mihara H, Esaki N, Pilon-Smits EAH (2003) Enhanced selenium tolerance and accumulation in transgenic *Arabidopsis thaliana* expressing a mouse selenocysteine lyase. Plant Physiol 131:1250–1257

Pilon-Smits EAH, Hwang S, Lytle CM, Zhu Y, Tai JC, Bravo RC, Chen Y, Leustek T, Terry N (1999) Overexpression of ATP sulfurylase in Indian mustard leads to increased selenate uptake, reduction, and tolerance. Plant Physiol 119:123–132

Pilon-Smits EAH, Quinn CF, Tapken W, Malagoli M, Schiavon M (2009) Physiological functions of beneficial elements. Curr Opin Plant Biol, in press

Quinn CF, Freeman JF, Galeas ML, Klamper EM, Pilon-Smits EAH (2008) Selenium protects plants from prairie dog herbivory – implications for the functional significance and evolution of Se hyperaccumulation. Oecologia 155:267–275

Rosenfeld I, Beath OA (1964) Selenium, geobotany, biochemistry, toxicity, and nutrition. Academic, New York

Smith FW, Ealing PM, Hawkesford MJ, Clarkson DT (1995) Plant members of a family of sulfate transporters reveal functional subtypes. Proc Natl Acad Sci USA 92:9373–9377

Sors TG, Ellis DR, Salt DE (2005) Selenium uptake, translocation, assimilation and metabolic fate in plants. Photosynth Res 86:373–389

Stadtman TC (1990) Selenium biochemistry. Annu Rev Biochem 59:111–127

Stadtman TC (1996) Selenocysteine. Annu Rev Biochem 65:83–100

Tamaoki M, Freeman JL, Pilon-Smits EAH (2008) Cooperative ethylene and jasmonic acid signaling regulates selenite resistance in *Arabidopsis thaliana*. Plant Physiol 146:1219–1230

Terry N, Zayed AM, de Souza MP, Tarun AS (2000) Selenium in higher plants. Ann Rev Plant Physiol Plant Mol Biol 51:401–432

Thompson-Eagle ET, Frankenberger WT Jr, Karlson U (1989) Volatilization of selenium by *Alternaria alternata*. Appl Environ Microbiol 55:1406–1413

Van Hoewyk D, Garifullina GF, Ackley AR, Abdel-Ghany SE, Marcus MA, Fakra S, Ishiyama K, Inoue E, Pilon M, Takahashi H, Pilon-Smits EAH (2005) Overexpression of AtCpNifS enhances selenium tolerance and accumulation in *Arabidopsis*. Plant Physiol 139:1518–1528

Van Hoewyk D, Abdel-Ghany SE, Cohu C, Herbert S, Kugrens P, Pilon M, Pilon-Smits EAH (2007) The *Arabidopsis* cysteine desulfurase CpNifS is essential for maturation of iron-sulfur cluster proteins, photosynthesis, and chloroplast development. Proc Natl Acad Sci USA 104:5686–5691

Van Hoewyk D, Takahashi H, Hess A, Tamaoki M, Pilon-Smits EAH (2008) Transcriptomeand biochemical analyses give insights into selenium-stress responses and selenium tolerance mechanisms in *Arabidopsis*. Physiol Plant 132:236–253

Van Huysen T, Abdel-Ghany S, Hale KL, LeDuc D, Terry N, Pilon-Smits EAH (2003) Overexpression of cystathionine-γ-synthase in indian mustard enhances selenium volatilization. Planta 218:71–78

Van Huysen T, Terry N, Pilon-Smits EAH (2004) Exploring the Selenium phytoremediationpotential of transgenic *Brassica juncea* overexpressing ATP sulfurylase or cystathionine γ-synthase. Int J Phytoremed 6:111–118

Unni E, Koul D, Alfred Yung W-K, Sinha R (2005) Se-methylselenocysteine inhibits phosphatidylinositol 3-kinase activity of mouse mammary epithelial tumor cells in vitro. Breast Cancer Res 7:R699–R707

Whanger PD (1989) China, a country with both selenium deficiency and toxicity: some thoughts and impressions. J Nutr 119:1236–1239

White PJ, Bowen HC, Marshall B, Broadley MR (2007) Extraordinarily high leaf selenium to sulfur ratios define 'Se-accumulator' plants. Ann Bot 100:111–118

White PJ, Broadley MR (2009) Biofortification of crops with seven mineral elements often lacking in human diets – iron, zinc, copper, calcium, magnesium, selenium and iodine. New Phytol 182:49–84

Wilber CG (1980) Toxicology of selenium: a review. Clin Toxicol 17:171–230

Wilson LG, Bandurski RS (1958) Enzymatic reactions involving sulfate, sulfite, selenate and molybdate. J Biol Chem 233:975–981

Yoshimoto N, Takahashi H, Smith FW, Yamaya T, Saito K (2002) Two distinct high affinity sulfate transporters with different inducibilities mediate uptake of sulfate in *Arabidopsis* roots. Plant J 29:465–473

Yoshimoto N, Inoue E, Saito K, Yamaya T, Takahashi H (2003) Phloem-localizing sulfate transporter, Sultr1;3, mediates re-distribution of sulfur from source to sink organs in *Arabidopsis*. Plant Physiol 131:1511–1517

Zayed AM, Terry N (1994) Selenium volatilization in roots and shoots: effects of shoot removal and sulfate level. J Plant Physiol 143:8–14

Zhang L, Byrne PF, Pilon-Smits EAH (2006a) Mapping quantitative trait loci associated with selenate tolerance in *Arabidopsis thaliana*. New Phytol 170:33–42

Zhang L-H, Ackley AR, Pilon-Smits EAH (2006b) Variation in selenium tolerance and accumulation among nineteen *Arabidopsis* ecotypes. J Plant Physiol 164:327–336

Zhang L-H, Abdel-Ghany SE, Freeman JL, Ackley AR, Schiavon M, Pilon-Smits EAH (2006c) Investigation of Selenium tolerance mechanisms in *Arabidopsis thaliana*. Physiol Plant 128:212–223