Chapter 10
Manipulating Selenium Metabolism in Plants: A Simple Twist of Metabolic Fate Can Alter Selenium Tolerance and Accumulation

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Abstract Selenium (Se) is a micronutrient for many organisms including humans. But like many trace elements, Se can be toxic at high concentrations and become a public health concern if it accumulates in soils or groundwater. Although higher plants don't require Se, plants can still accumulate and metabolize Se *via* the sulfur assimilatory pathway. Genetic manipulation of plant selenium metabolism primarily stems from two areas of interest: it has the potential to improve the phytoremediation of Se in contaminated areas, and it may aid the development of Se-containing phytochemical compounds that possess health benefits. This review highlights studies that have successfully altered Se metabolism in plants, and concludes by focusing on novel genes and pathways that might be targeted to manipulate Se metabolic processes.

Keywords Selenium • Metabolism • Oxidative stress • Selenoprotein • miRNA

10.1 Introduction

Selenium (Se) is an essential trace element for mammals, bacteria, and some green algae (Stadtman 1996). However, it is unlikely to be required by higher plants even though it can be beneficial (El Mehdawi and Pilon-Smits EAH 2012; Feng et al. 2013). As a nutrient in humans, Se is an essential component of the 21st amino acid selenocysteine, which is used to make 25 selenoproteins (Papp et al. 2007). A daily intake of 55 micograms of Se is recommended (Institute of Medicine 2000).

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A deficiency in dietary Se decreases the abundance of selenoproteins, and can lead to Kashin-Beck and Keshan disease, which alters bone and cardiac function, respectively. Additionally, numerous *in vitro* studies have reported the protective properties of Se compounds, particularly against cancer (Davis 2012). Due to its anticarcinogenic properties, Se supplementation or Se-fortified crops may be beneficial, but this is still controversial.

While Se levels in most soils are between 0.01 and 2 Se/kg, Se naturally accumulates in certain Cretaceous shale sediments. Such seleniferous soils can contain up to 100 mg/kg Se (Pilbeam et al. 2015). Anthropogenic activities, such as irrigation, can also result in Se accumulation in soil and potentially crops (Zhu et al. 2009). Selenium is chemically very similar to S, and its inadvertent accumulation in plants occurs primarily when selenate is transported into roots via sulfate transporters (White 2015). Selenate is readily translocated into shoot tissue, where is can be metabolized by chloroplastic enzymes involved in S assimilation.

Se decreases growth of most plants at concentrations exceeding 10–25 μM (Zhang et al. 2006). In non-hyperaccumulating plants- including most crops- Se toxicity occurs if its foliar accumulation exceeds 10–100 μg, as recently reviewed (White 2015). Selenium toxicity stems from two separate processes (Van Hoewyk 2013). Inorganic Se, particularly selenite, can redox cycle with thiols and generate reactive oxygen species (Spallholz 1994), including hydrogen peroxide (Tamaoki et al. 2008) and mitochondrial superoxide that alters respiration (Dimkovikj and Van Hoewyk 2014). Additionally, Se is likely toxic when it replaces S in protein. This hypothesis originated by the discovery that Se-tolerant *Astragalus* species were found to have nearly tenfold lower concentration of Se in protein compared with non-tolerant *Astragalus* species (Brown and Shrift 1981). In particular, the substitution of cysteine with selenocysteine (Sec) is believed to cause protein misfolding (Stadtman 1990). Several lines of evidence bolster the hypothesis that Se causes protein misfolding. Selenocysteine causes severe toxicity in Arabidopsis plants with a mutation in Bip2 (Sabbagh and Van Hoewyk 2012), an endoplasmic reticulum protein that participates in the unfolded protein response and renders mutant plants sensitive to agents that cause protein misfolding. Arabidopsis plants with mutations in ER protein quality control are also sensitive when treated with selenate (Van Hoewyk 2016). Additional support for the malformed selenoprotein hypothesis comes from the observation that selenoproteins can be removed by the ubiquitin-proteasome pathway in a variety of plants, including the Se-hyperaccumulator *Stanleya pinnata* (Sabbagh and Van Hoewyk 2012), canola (Dimkovikj et al. 2015), and the green algae *Chlamydomonas* (Vallentine et al. 2014).

Averting Se toxicity may potentially improve efforts to clean polluted soils and water *via* phytoremediation (Pilon-Smits 2005). Additionally, the development of crops with fortified levels of Se is appealing, as a source of both nutrition and Se-based therapeutics. Therefore, in some circumstances it may be desirable to use plants more efficiently for phytoremediation or as Se-fortified foods. To meet this aim, several different plant genetic engineering strategies have been designed and used successfully to further enhance plant Se metabolism, including its uptake and

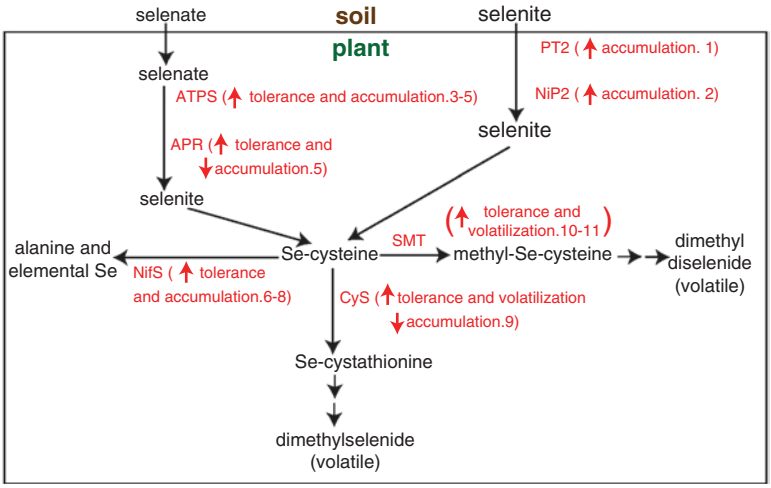

Fig. 10.1 Schematic diagram highlighting transgenic approaches that have altered selenium metabolism in plants. *Black*: Se metabolites; *Red*: manipulated enzymes and their impacts on selenium metabolism

accumulation, volatilization, and tolerance (Pilon-Smits and LeDuc 2009). Most genetic engineering approaches have targeted enzymes participating in S uptake or assimilation. However, a few studies have manipulated Se metabolism by focusing on genes unrelated to S metabolism, and there is compelling evidence that other unidentified pathways may also control plant Se tolerance and accumulation. These strategies are discussed below and summarized in Fig. 10.1.

10.2 Targeting Sulfur Transporters Alters Selenium Accumulation in Plants

In terrestrial ecosystems, selenate is the most bioavailable form of Se in soil (Terry et al. 2000). Although a selenate-specific transporter in plants remains elusive, it can be transported into roots *via* sulfate transporters. Mutation of sulfate transporter SULTR1;2 in Arabidopsis improved selenate tolerance by restricting selenate entry into the plant, and therefore decreased Se accumulation; mutations in ten other sulfate transporters did not affect selenate tolerance (El Kassis et al. 2007). Although it remains to be confirmed, overexpression of SULTR1;2 would likely increase Se accumulation in crops, but comes with the caveat that that increased Se content may also potentially decrease Se tolerance.

In aquatic ecosystems or flooding conditions that promote anaerobia, selenite is likely to be the predominant Se metabolite available for plant uptake. Similar to selenate, a selenite-specific transporter has yet to be identified. However, overexpression of phosphate transporter (OsPT2) in rice increased selenite uptake and Se

accumulation in rice grains (Zhang et al. 2014). Additionally, a silicon transporter in rice (OsNiP2;1) appears to assist in selenite transport under acidic conditions (Zhao et al. 2010). Lastly, in addition to transporters, exogenous glutathione (GSH) can increase selenite transport in rice (Zhang 2015), indicating that perhaps GSH content in roots can control selenite uptake. Whether or not genetic engineering of GSH content in roots can augment selenite transport and accumulation remains to be determined.

10.3 Manipulation of the Sulfate Reduction Pathway Alters Metabolism

The sulfate reduction pathway controls the flux of the assimilation of sulfate into cysteine, as extensively reviewed (Çakır et al. 2012; Hawkesford and De Kok 2006; Pilon-Smits 2015; White 2015). Given that Se and S behave similarly, initial attempts aimed at manipulating Se metabolism have targeted enzymes involved in sulfate assimilation. The reduction of sulfate to sulfide occurs in plastids and involves the concerted actions of ATP sulfurylase (ATPS), adenosine 5-phosphoreductase (APR), and sulfite reductase (SiR). The reduction of selenate to selenite is likely a rate-limiting step for the assimilation of selenate into organic Se. This conclusion is based on studies reporting that plants treated with selenate accumulated mainly selenate, while plants that were fed selenite accumulated mainly organic Se (de Souza et al. 1998; Zayed et al. 1998). To overcome this apparent rate limitation in Se metabolism, Arabidopsis APTS- which activates sulfate- was overexpressed in *Brassica juncea* (Pilon-Smits et al. 1999). When treated with selenate, these transgenic plants accumulated an organic form of Se, in contrast to wild-type plants that accumulated selenate. Although Se volatilization was unaltered, the ATPS transgenics were more tolerant to selenate and accumulated threefold to fivefold more Se than wild type in both laboratory and in the field (Bañuelos et al. 2005); this phenotype was explained by their ability to quickly metabolize inorganic Se into organic forms. However, an alternative explanation to their improved Se tolerance may also be envisioned. When ATPS was overexpressed in Arabidopsis, it also resulted in increased Se accumulation and assimilation of organic Se, but was also accompanied by increased levels of cysteine and GSH (Sors et al. 2005). Elevated levels of GSH can maintain redox poise during oxidative stress (Noctor et al. 2012), and is associated with improved Se tolerance (Grant et al. 2011). Therefore, it is possible that improved Se tolerance in ATPS transgenics could have at least partially stemmed from an elevated GSH status.

APR catalyzes the reaction of activated sulfate to sulfite. When APR from *Pseudomonas aeruginosa* was overexpressed in Arabidopsis, it also increased the proportion of organic Se and improved tolerance when treated with selenate (Sors et al. 2005). Although an Arabidopsis APR isoform has not been overexpressed, knockout of APR in Arabidopsis was associated with decreased Se accumulation

and tolerance, which was explained by the observed decrease in glutathione and superoxide accumulation (Grant et al. 2011). Taken together, these data indicate that APR also controls the flux of selenate into organic forms, similar to ATPS.

Sulfite is converted into sulfide *via* sulfite reductase (SiR), but it is doubtful that the enzyme also has selenite reductase activity (Ng and Anderson 1979). Rather, GSH likely non-enzymatically reduces selenite to selenide, and in doing so generates superoxide (Seko et al. 1989; Kessi and Hanselmann 2004). Additionally, Arabidopsis plants with decreased levels of SiR do not display altered tolerance when stressed with selenite (Fisher et al. 2016), suggesting that knockdown of SiR does not play an important role in determining Se tolerance or accumulation.

10.4 Minimizing Se-Cysteine Incorporation in Protein Improves Se Tolerance in Plants

Astragalus bisulcatus' tolerance to Se is attributable to the presence of a chloroplastic enzyme with selenocysteine methyltransferase (SMT) activity (Neuhierl and Bock 1996). This enzyme methylates Sec and prevents its incorporation into protein; therefore, the formation of malformed selenoproteins is avoided. Methyl-Sec is the predominant Se-containing metabolite in Se hyperaccumulators (Whanger 2002). *SMT* has been cloned and characterized from different plant species (Cakir and Ari 2013; Lyi et al. 2005; Neuhierl and Bock 1996; Sors et al. 2009; Zhu et al. 2008), and it is widely believed that this enzyme confers Se tolerance in Se-hyperaccumulating plants. Methyl-Sec can be further metabolized to non-toxic dimethyl-diselenide, a volatile molecule that is emitted into the atmosphere (de Souza et al. 1998). The *A. bisulcatus* SMT enzyme has been overexpressed in *A. thaliana* and *B. juncea* (Ellis et al. 2004; LeDuc et al. 2004). In both species, selenite-treated SMT-transgenic plants converted Sec to methyl-Sec. The ability to convert Sec to methyl-Sec was associated with increased total Se accumulation, improved Se tolerance, and enhanced volatilization of dimethyl-diselenide. The non-hyperaccumulator *Astragalus drummondii* also possesses an *SMT*-like gene (Sors et al. 2009). Despite its homology to the gene from *A. bisulcatus*, biochemical studies revealed that the enzyme from *A. drummondii* lacks SMT activity, thus likely rendering the plant intolerant to Se. Mutagenesis of the *A. drummondii* gene to make it more similar to the one from *A. bisulcatus* provided some SMT activity, but still the mutated enzyme was not as active as its homologue in *A. bisulcatus* (Sors et al. 2009). Additionaly, *B. juncea* over-expressing both APS and SMT increased Se accumulation up to ninefold compated to WT plants (LeDuc et al. 2006). Collectively, these experimental studies reveal that SMT activity plays a vital role in Se hyperaccumulation, and *A. bisulcatus* SMT provides both increased Se tolerance and accumulation when genetically engineered in non-hyperaccumulators. This may ultimately prove useful for the environmental cleanup of seleniferous soils or to fulfill the human dietary needs of Se.

Cystathionine gamma synthase (CgS) can also prevent the formation of non-specific selenoproteins by catalyzing the reaction of Sec to seleno-cystathionine, a precursor metabolite of Se-methionine. Overexpression of Arabidopsis *CgS* in *B. juncea* improved Se tolerance, which was explained by a twofold to threefold increase in Se volatilization (Van Huysen et al. 2003). As a result of enhanced volatilization, the CgS transgenics accumulated 40% less Se compared to wild-type plants. These results indicate that CgS is involved and rate limiting in Se volatilization.

In another approach to divert Sec from being incorporated into proteins, genetic engineering approaches have also targeted Sec-lyases, which catabolize Sec into alanine and elemental Se. Initially, a mouse Sec-lyase was over-expressed in Arabidopsis, which decreased the amount of Se in protein, yet increased Se accumulation (Pilon et al. 2003). Overexpression of Sec-lyase in the cytosol improved Se tolerance, but intriguingly, targeting of this enzyme to the chloroplast increased sensitivity to Se. This could potentially be explained by the ability of elemental Se to replace S in chloroplastic Fe-S proteins. Fe-Se clusters are known to be unstable and their incorporation into proteins can decrease activity (Hallenbeck et al. 2009). Sequencing of the Arabidopsis genome revealed a chloroplastic Sec-lyase called CpNifS. Overexpression of CpNifS in Arabidopsis increased Se accumulation and selenate tolerance almost twofold, and this phenotype was associated with a 33% decrease of Se in protein and increased S levels (Van Hoewyk et al. 2005). Additionally, *B. juncea* over-expressing a Sec-lyase also accumulated Se twofold when grown in soil polluted with Se (Bañuelos et al. 2007). In summary, these data indicate that overexpression of CpNifS prevents the formation of selenoproteins in plants, which likely explains their improved tolerance to selenate.

10.5 Manipulation of Oxidative Stress Response Genes Alters Se Metabolism

As mentioned above, Se is known to induce oxidative stress in plants. Thus, antioxidant systems may contribute to plant Se tolerance. Indeed, several studies have indicated that overexpression of genes associated with an oxidative stress response improve Se tolerance and alter plants' ability to accumulate Se.

Arabidopsis selenium-binding protein (*SBP1*) was the first gene unrelated to sulfur metabolism whose overexpression improved Se (Agalou et al. 2005). Expression of this gene is tightly linked to oxidative stress, and is also induced during sulfur starvation. Although its biological function remains unknown, SBP1 has been speculated to have antioxidant properties (Hugouvieux et al. 2009), as its overexpression in Arabidopsis also improves tolerance to cadmium and hydrogen. However, recently it was discovered that SPB1 can bind to a variety of heavy metals; additionally, it can bind to and reduce selenite, but not selenate (Schild et al. 2014). Therefore, increased tolerance in SBP1 transgenics may also be attributed to its capacity to prevent selenite-induced oxidative stress that can impair mitochondrial function

(Dimkovikj and Van Hoewyk 2014). In agreement with this conclusion, human cells with mutant SBP1 are sensitive to selenite and suffer from mitochondrial damage (Ying et al. 2015).

The story of a broccoli methyltransferase (*BoCOQ5–2*) expressed in Arabidopsis further demonstrates that manipulating Se metabolism can be achieved by targeting pathways independent of sulfur metabolism (Zhou et al. 2009). BoCOQ5–2 is involved in the biosynthesis of ubiquinone, which has a role in respiration; additionally, it is an antioxidant in plants (Ohara et al. 2004) and likely protects mitochondria during stress (Bergamini et al. 2012). Transgenic COQ5–2 plants had improved Se tolerance, which was associated with decreased levels of hydrogen peroxide and increased dimethyl diselenide volatilization. Ubiquinone levels were not elevated in these plants. The authors conclude that increased volatilization was unlikely to be a direct consequence of manipulating the ubiquinone pathway. Rather, increased dimethyl diselenide volatilization likely stemmed from an improved antioxidant status in the COQ5–2 plants. If that is the case, then it is possible that increased levels of other antioxidants- such as vitamin C and vitamin E- may also alter Se metabolism in plants (Zhou and Li 2010). In line with the hypothesis that improved oxidative stress tolerance can alter Se metabolism in plants, overexpression of GSH synthetase also increases Se tolerance and accumulation in *B. juncea* (Bañuelos et al. 2005). In Arabidopsis, tolerance to selenite correlates tightly with internal GSH concentrations (Grant et al. 2011). More recently, overexpression of a peroxidase implicated in drought and salt stress also protected Arabidopsis plants against Se (Jiang et al. 2015). In summary, Se metabolism can be altered by genetic engineering approaches aimed at improving oxidative stress tolerance.

10.6 Transcriptomics Reveal Additional Genes That May Alter Se Metabolism and Tolerance

The advent of high-throughput sequencing has allowed researchers to identify genes and pathways responsive to stress conditions. For example, the transcriptome of selenate-treated Arabidopsis revealed an upregulation of many transcripts involved in ethylene and abscisic acid synthesis and signaling (Van Hoewyk et al. 2008). Indeed, further genetic analysis demonstrated that decreased levels of these two hormones increase both selenate and selenite sensitivity (Tamaoki et al. 2008), likely by mediating an oxidative stress response. A more recent transcriptome study used RNA-seq to determine the effects of selenate in *Astragalus chrysochlorus*, a secondary Se accumulator (Çakır et al. 2015). This study revealed an upregulation of genes involved in ABC transport, plant pathogen interactions, and biosynthesis of secondary metabolites. Additionally, many putative transcription factors were upregulated, including: TCP13-like, bZIP, bHLH041-like, heat stress A-3-like, trihelix GT-3b-like, and WRKY32. Additional experimentation is needed to elucidate if manipulation of these identified genes play a role in Se tolerance and accumulation.

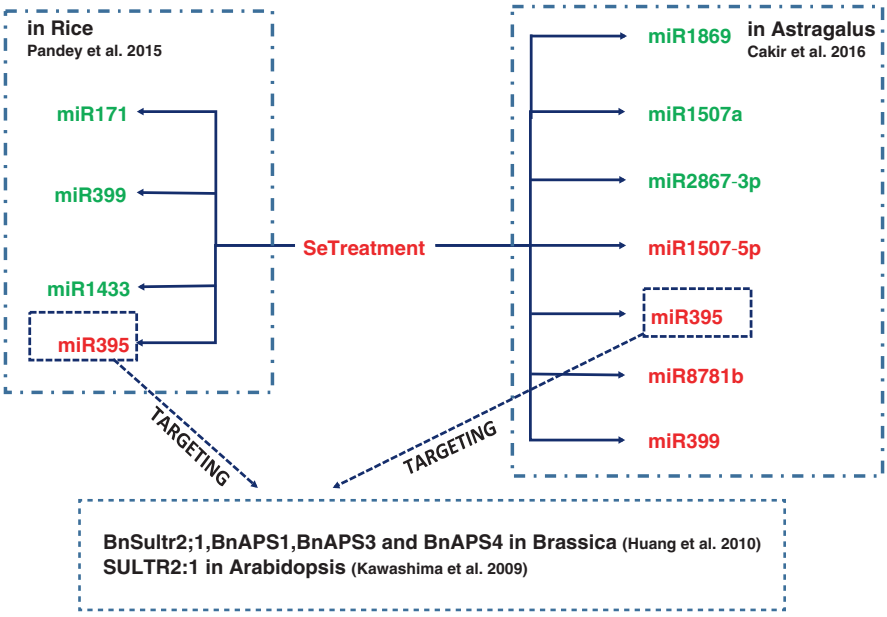

Fig. 10.2 Se treatment in plants alters the expression of miRNAs. *Green*: up-regulation. *Red*: down-regulation

Increased GSH concentration in plants is associated with improved tolerance to agents that induce oxidative stress, including Se (Noctor et al. 2012; Grant et al. 2011). Optimal glutamate and glutathione metabolism in Arabidopsis plants is maintained by glutamyl cyclotransferase (GGCT2; 1); this enzyme participates in the glutamyl cycle by recycling glutamate from GSH-conjugates, which can subsequently be used to make new GSH. Overexpression of GGCT2;1 in Arabidopsis improved arsenate tolerance; this phenotype was explained by the increased cytosolic breakdown of GSH conjugated to arsenic and decreased demand of *de novo* glutamate generated by the TCA cycle (Paulose et al. 2013). Selenate-treatment has been reported to decrease glutamate concentration in Arabidopsis (Van Hoewyk et al. 2008; Grant et al. 2011). It is possible that GGCT2;1 transgenics also confer Se tolerance, as suggested by a transcriptome study (Van Hoewyk et al. 2008). GGCT2;1 mRNA increased almost 100-fold in selenate-treated Arabidopsis (Van Hoewyk et al. 2008). Additionally, GGCT2;1 protein increases in *B. napus* treated with selenite (Dimkovikj and Van Hoewyk 2014), further implicating its involvement in a Se-stress response. Future studies may reveal that GGCT2;1 overexpression alleviates Se toxicity.

MicroRNAs (miRNAs) have also been recently implicated in mediating a Se response, as depicted in Fig. 10.2. Noncoding miRNAs post-transcriptionally regulate gene expression by participating in the degradation of target mRNAs (Bartel 2004), thereby inhibiting translation. miRNAs are known to function in many developmental and physiological processes (Zhang and Wang 2015). Two recent studies

have explored how Se affects miRNA expression in plants. In one study, Se-induced miRNAs were identified in *A. chrysochlorus* using next generation sequencing analysis (Çakir et al. 2016). Computational studies revealed that Se induced miRNAs that target mRNAs controlling hormone signaling, plant-pathogen interactions, and sulfur metabolic pathways. The most significantly affected miRNAs were miR1507a, miR1869 and miR2867-3p, miR1507-5p and miR8781b; however, it is unknown what these miRNAs target or how they might mediate Se tolerance and accumulation. In another study performed in rice, Se increased expression of miR171, miR399 and miR1433, but decreased expression of miR395 (Pandey et al. 2015). miR395 targets ATP sulfurylases ATPS1 and ATPS4 and the sulfate transporter SULTR2;1 (Kawashima et al. 2009; Huang et al. 2010). These genes control sulfate accumulation and assimilation, and their expression was inversely correlated with decreased miR395 expression in rice. This result nicely coincides with transcriptome studies in Arabidopsis demonstrating that selenate induces genes involved in sulfur transport and assimilation (Van Hoewyk et al. 2008). In summary, manipulating miRNAs may also provide new approaches to alter Se metabolism.

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