Chapter 16
Manipulation of Selenium Metabolism in Plants for Tolerance and Accumulation

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16.1 Introduction

Selenium (Se) is an essential element for many life forms. Humans, in particular, require trace Se as a key component of selenocysteine (SeCys), which is recognized as the 21st protein amino acid and is typically embedded at the catalytic site of 25 known selenoproteins (Labunsky et al. 2014; Carlson et al. 2018). Human selenoproteins exhibit a wide range of functions in cellular metabolism, resulting in critical health effects. In particular, they favor the immune system and defense mechanisms against free radicals and oxidative stress and regulate thyroid metabolism and spermatogenesis (Rayman 2020; Lima et al. 2021).

The United States Recommended Dietary Allowance for Se in adults is 55–75 μg/day (National Academy of Sciences 2000), while the threshold of chronic Se intake that might cause toxicity is 400 μg/day (Institute of medicine 2000; Vinceti et al. 2018). Although cases of chronic selenosis, i.e., the condition caused by excessive Se, were documented in Enshi, (China) (Huang et al. 2013) and in Punjab (India) (Hira et al. 2004; Chawla et al. 2020), Se deficiency is a more frequent condition, and the number of people who are suffering from it is increasingly growing on a global scale (Jones et al. 2017). Se deficiency manifests subtly as augmented susceptibility to viral infections, cancers, and other diseases (Lima et al. 2021; Schiavon et al. 2020). Acute selenosis can also occur in humans and is generally caused by the ingestion of Se-rich chemical products and excessive amounts of dietary Se supplements (MacFarquhar et al. 2010).

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Plants represent a major source of Se to humans, and therefore Se consumption ultimately depends on the concentration of available Se in soil, as well as on the plant capacity to take up and convey Se to the aboveground produce (Yang et al. 2021). Globally, Se concentration in soils is generally very low, but a few soils termed "seleniferous" naturally contain elevate amounts of Se (more than 100 mg Se/kg). Other soils are high in Se due to anthropogenic Se contamination and need reclamation (Khamkhash et al. 2017).

Intriguingly, plant do not require Se for their growth and metabolism, but this element at low concentration can induce several beneficial effects in them, such as a greater growth and resistance against abiotic and biotic stress (Chauhan et al. 2019). At high concentration, however, Se generates toxicity in plants causing protein misfolding or acting as a prooxidant agent through the redox cycling with thiols and by producing reactive oxygen species (Van Hoewyk 2013; Kolbert et al. 2019).

Plants do not appear to possess specific mechanisms for Se uptake; thus, Se is likely transported across cell membranes nonspecifically through the action of transport proteins for other nutrients (Trippe and Pilon-Smits 2021). Though, certain plants, termed Se hyperaccumulators, are believed to own specific transporters that assist Se uptake and are hypothesized to be responsible for their extraordinary ability to accumulate Se in their shoot (>1000 mg Se/kg) when growing in seleniferous soils (White et al. 2016, 2018). Also, Se hyperaccumulators have evolved specific biochemical pathways that allow them to avert Se toxicity and whose metabolites serve ecological functions (Pilon-Smits 2019). On this account, Se-hyperaccumulators offer a fascinating genetic pool for the selection of candidate genes to transfer to non-hyperaccumulators through genetic engineering. So far, sulfur-related proteins that mediate Se transport or are involved in Se assimilation or detoxification (including volatilization) have been overexpressed in non-hyperaccumulators using different manipulation strategies, resulting in enhanced capacity of plants to accumulate and tolerate Se (Pilon-Smits and LeDuc 2009). However, as the development of Se-enriched plants has become particularly attractive for both phytoremediation and biofortification scopes, this requirement has prompted research to identify novel molecular targets unrelated to S transport and metabolism.

16.2 Genetic Engineering of Se Accumulation and Tolerance

16.2.1 Manipulation of Selenium Transport

Among Se species present in terrestrial ecosystems, selenate is the most available in oxic soils, while selenite dominates under anoxic conditions, as those in flooded soils (Winkel et al. 2015; Shahid et al. 2018). Organic selenocompounds can be appreciably present in seleniferous soils and derive mainly from the decomposition of Se-hyperaccumulator litter (Pilon-Smits 2019). Once in the soil, these

compounds, especially the Se-amino acids, can be readily taken up by plants thus contributing to Se cycling (Winkel et al. 2015).

Plants take up both selenate and selenite ions, but neither ion can cross the root cell membranes through Se-specific transporters. Selenate, in particular, makes use of sulfate transporters (SULTRs) for the entry into cells owing to its chemical similarity to sulfate (Schiavon and Pilon-Smits 2017a; Trippe and Pilon-Smits 2021). However, the hypothesis that other transporters beside SULTRs may be responsible for selenate movement within the plant cannot be excluded.

SULTR1;2 is the major root high-affinity transporter involved in the primary sulfate uptake. The mutation of SULTR1;2 in *Arabidopsis* resulted in enhanced Se tolerance through restriction of selenate uptake and accumulation in the plant, but mutations in other sulfate transporters did not appear to modulate Se tolerance (El Kassis et al. 2007). The Se-hyperaccumulator *Stanleya pinnata* has a very high expression of the gene encoding SpSULTR1;2 (Wang et al. 2018), which, contrary to non-hyperaccumulators, is not subjected to the canonical feedback regulation operated either by the availability of sulfate or the S state of the plant (El Mehdawi et al. 2018). Therefore, SpSULTR1;2 could have higher specificity for Se over S, which would explain the high Se/S ratios observed in *Stanleya pinnata* and, perhaps, the S-independent seasonal variation in Se concentration in different plant organs of different Se-hyperaccumulators. If this will be corroborated, the transfer of *SpSultr1;2* into crops or high biomass plants would have the potential to increase their Se storage capacity, thus greatly improving the effectiveness of biofortification and phytoremediation programs, respectively. Overexpression of *Sultr1;2* transporter from non-hyperaccumulators could also result in higher rates of Se uptake in transgenic plants, even though competition with sulfate will be more significant at the root surface.

Selenite entry into the plants is also assisted by nonspecific mechanisms including aquaporins (e.g., NIP2.1, Lsi1) and phosphate transporters (Zhao et al. 2010a, b; Zhang et al. 2014; Schiavon and Pilon-Smits 2017a). The overexpression of the phosphate transporter OsPT2 in rice conferred a greater uptake capacity of selenite with a consequent increase in the accumulation of Se in the rice grains (Zhang et al. 2014). Furthermore, glutathione (GSH) applied to rice plants was effective in promoting the selenite transport (Zhang et al. 2015). This observation makes it plausible that genetic engineering of GSH content in the roots can be applied to control the absorption of selenite, but the hypothesis remains to be ascertained. Since selenite is the prevalent form of Se available in paddy fields where rice plants are commonly grown, understanding how plants take up selenite from anoxic soils will be useful for rice engineering in regions where dietary Se is low and mainly relies on this crop as the main food source.

I should be noted that increasing the accumulation of Se in plants by enhancing their capacity to absorb Se, both in the case of selenate and selenite, carries the risk that plants may not tolerate a high Se concentration in their cells. Reduced selenium tolerance often results in a drop in plant biomass and a reduction in productivity, which is not an intended event.

Once absorbed, selenite is readily assimilated within cells, whereas selenate can move in the plant likely through the assistance of sulfate transporters (El-Mehdawi et al. 2018; White et al. 2004, 2016). The gene encoding the low-affinity sulfate transporter *Sultr2;1* that is responsible for sulfate loading into the xylem was found to be largely more expressed in *S. pinnata* than in non-hyperaccumulators, thus leading to the hypothesis that perhaps it explains the exceptional accumulation of Se in the hyperaccumulator shoot (El Mehdawi et al. 2018).

In addition to *Sultr 1;2* and *Sultr2;1*, other *Sultr* genes could be considered potential targets of genetic engineering, such as those involved in sulfate/selenate entry into plastids (SULTR3 group), or in sulfate/selenate translocation into sink organs, like *Sultr1;3*. In this regard, the study by Wang et al. (2018) offers a nice overview of different sulfate transporters upregulated in the leaves and roots of *S. pinnata* compared to the related non-hyperaccumulator *S. elata* that could be investigated.

The same study revealed the extraordinary expression of a gene encoding the amino acid transporter LYSINE HISTIDINE TRANSPORTER1 (LHT1), a homolog of amino acid permease (AAP), in roots of *S. pinnata*. It is reasonable that Se-amino acids, either methylated or not, can enter the root cells by engaging amino acid permeases with broad substrate specificity (Schiavon et al. 2020). This hypothesis arises from the evidence that proline competes with cysteine (Cys) and methionine (Met) for the uptake by the plant (Frommer et al. 1993), and some plants like durum wheat (*Triticum turgidum*) and spring canola (*Brassica napus*) show a preference for Se-amino acids over inorganic Se (Zayed et al. 1998; Kikkert and Berkelaar 2013). At present, LHT1 is under investigation to confirm its function in the transport of Se-amino acids.

Very recently, it has been reported that the transporter NRT1.1B belonging to the family of peptide transporters (PTRs) that assists nitrate transport also manifests the transport capacity of SeMet (Zhang et al. 2019). Consistently, NRT1.1B overexpression in rice plants was associated with higher SeMet loading into the grains. This gene could therefore be a particularly interesting target for biofortification.

A summary of the main studies on Se transport is reported in Table 16.1.

16.2.2 Manipulation of Genes Implied in Selenate Reduction

Being similar to sulfate, selenate is assimilated along the S pathway in plastids to be converted into Se-amino acids. Therefore, the first attempts to manipulate Se metabolism targeted enzymes that function in sulfate assimilation. The first reaction of the process involves the activation of sulfate/selenate to adenosine 5′-phosphosulfate/selenate (APS/APSe) by the enzyme adenosine triphosphate sulfurylase (APS) (Bohrer et al. 2015). This step is considered to be limiting for selenate assimilation (White et al. 2016, 2018; Lima et al. 2018), and the overexpression of adenosine triphosphate sulfurylase isoform 1 (APS1) in *Brassica juncea* and *A. thaliana* has successfully overcome this limitation by promoting the reduction of selenate to

Table 16.1 List of transporters in involved Se uptake for different plant species

Plant species	Transporters	Se species	Reference(s)
Arabidopsis thaliana L.	SULTR1;2,	Selenate	El Kassis et al. (2007)
Astragalus racemosus (HA), *Astragalus bisulcatus* (HA), *Astragalus glycyphyllos* (n-HA), *Astragalus drummondii* (n-HA)	SULTR group 1, 2 and 4	Selenate	Cabannes et al. (2011)
Brassica juncea L. Czern. (n-HA), *Stanleya elata* L. (n-HA), *Stanleya Pinnata* L. (HA)	SULTR1;1, SULTR1;2, SULTR 2;1	Selenate	Schiavon et al. (2015), Wang et al. (2018) and El Mehdawi et al. (2018)
Oryza sativa L. (n-HA)	NIP2;1	Selenite	Zhao et al. (2010a, b)
Oryza sativa L. (n-HA)	(OsPT2)	Selenite	Zhang et al. (2014)
Triticum aestivum L. (n-HA)	SULTR1;1, SULTR4;1	Selenate	Shinmachi et al. (2010)
Oryza sativa L. (n-HA)	NRT1.1B	SeMet	Zhang et al. (2019)
Eruca sativa mill. (n-HA), *Diplotaxis tenuifolia* (n-HA)	SULTR1;1, SULTR1;2, SULTR 2;1	Selenate	Dall'Acqua et al. (2019)

n-HA non-hyperaccumulators, *HA* hyperaccumulators

APSe (Pilon-Smits et al. 1999). Transgenic *Brassica juncea* plants, in particular, when treated with selenate contained more organic Se than wild-type plants, which conversely accumulated more selenate (Pilon-Smits et al. 1999). Interestingly, these transgenics showed superior Se tolerance than wild type, although accumulated two- to three-fold more Se, possibly because they assimilated Se more easily. In *A. thaliana*, the overexpression of APS1 led to increased amounts GSH and its precursor cysteine (Sors et al. 2005). High levels of GSH are critical in antioxidative processes (Noctor et al. 2018; Hasanuzzaman et al. 2019) and may also explain greater tolerance of transgenics to Se (Grant et al. 2011).

More recently, the adenosine triphosphate sulfurylase isoform 2 (APS2) of *S. pinnata* has been identified as a new potential target of genetic engineering (Jiang et al. 2018). APS2 has both plastidial and cytosolic localization in non-Se hyperaccumulators *A. thaliana* and *Stanleya elata*, while only cytosolic in *S. pinnata* (Bohrer et al. 2015; Jiang et al. 2018). A transcriptomic study revealed amazing levels of Aps2 gene transcripts in *S. pinnata* compared to *S. elata* and predicts the hypothesis that high APS2 expression may be responsible for the Se hypertolerance trait typical of the hyperaccumulator.

In the later stages of S/Se assimilation, reactions are driven by the action of adenosine 5-phosphoreductase (APR) and sulfite reductase (SiR). APR converts APS/APSe to sulfite/selenide (White et al. 2018). Similar to APS, APR is supposed to be critical in controlling the assimilatory flow of selenate into Se-amino acids. The overexpression of APR from *Pseudomonas aeruginosa* in *A. thaliana* resulted in an increase in tolerance to Se and accumulation of organic Se (Sors et al. 2005). Also, APR knockout mutants of *A. thaliana* showed a decrease of their ability to accumulate and tolerate Se, likely because of the concurrent reduction of GSH levels and increase of reactive oxygen species (ROS) generation (Grant et al. 2011).

Regarding SiR, preliminary studies have not highlighted a key role for this enzyme in the tolerance and accumulation of Se, as neither overexpression nor knockdown of SiR did not produce any specific phenotype in *A. thaliana*. Therefore, SiR does not appear to be a potential candidate for the genetic engineering of plants to be enriched in Se.

16.2.3 Manipulation S-Related Genes for Averting Se-Amino Acid Incorporation into Proteins

As previously mentioned, Se-hyperaccumulating plants have evolved specific biochemical pathways to thrive in seleniferous soils and become hypertolerant to high Se concentrations accumulated in their tissues. In one of these pathways, the enzyme selenocysteine methyltransferase (SMT) plays a noteworthy role (Schiavon et al. 2017a; Chen et al. 2019). SMT prevents SeCys misincorporation into proteins via its methylation into methyl-selenocysteine (MetSeCys), therefore significantly reducing toxicity stemming from the generation of malformed proteins. MetSeCys is the dominant Se form in Se-hyperaccumulators, while non-hyperaccumulators mainly contain selenate or selenomethionine (SeMet) (Schiavon et al. 2017a; White et al. 2016, 2018). However, SMT activity was also determined in some Se-accumulators, such as broccoli (Lyi et al. 2005), and MetSeCys was quantified in broccoli, radish, rice, potato, and carrot (Amato et al. 2020). In Se non-hyperaccumulator *Astragalus drummondii*, although the SMT gene was identified, SeCys methylation activity was though absent (Sors et al. 2009). Induced mutation of the SMT gene in *A. drummondii* provided some SMT activity, but the mutated enzyme was not yet as active as its counterpart in the hyperaccumulator *A. bisulcatus* (Sors et al. 2009). Recently, a novel SMT has been identified in *B. juncea*, which can methylate both homocysteine and SeCys substrates (Chen et al. 2019). Overexpression of this enzyme in tobacco plants increased the accumulation of total Se and MeSeCys (Chen et al. 2019). Thus, the gene encoding SMT attains great attention as an appealing candidate for the genetic engineering of staple crops with fortified levels of Se. In fact, an increase in the synthesis of MetSeCys in the edible produce is a desirable trait because this metabolite is a reservoir of methylselenic acid which in humans determines greater resistance to certain types of cancer (Lima et al. 2021). The formation of MetSeCys can be a relevant metabolic step to be transferred also to the plants employed for Se phytoremediation, because MetSeCys can be further metabolized to volatile nontoxic dimethyldiselenide (DMDSe) dispersed in the air (White et al. 2018; Chauhan et al. 2019). On this account, the SMT gene has been cloned and characterized from different plant species (Çakir and Ari 2013; Lyi et al. 2005; Zhu et al. 2009; Neuhierl and Bock 1996; Sors et al. 2009). For example, SMT from the Se hyperaccumulator *Astragalus bisulcatus* was cloned and overexpressed in *A. thaliana* and *B. juncea* plants, which produced MetSeCys after being fed with selenite (Ellis et al. 2004; LeDuc et al. 2004). In addition, both

transgenics contained more total Se but were more tolerant to Se than the wild-type plants due to increased volatilization rates of Se in the form of DMDSe.

Another gene belonging to the S/Se metabolism that has been tested for its potential in improving the tolerance to Se while decreasing the accumulation of Se amino acids responsible for proteins misfolding is the one that codes for the enzyme cystathionine-β-synthase (CβS). This enzyme converts SeCys to seleno-cystathione, which is the substrate for the synthesis of SeMet. When CβS from *A. thaliana* was overexpressed in *B. juncea*, plants exhibited higher Se volatilization rates (at least two- to threefold than the wild type) and greater tolerance to Se (van Huysen et al. 2003), therefore confirming that SeCys conversion to seleno-cystathione is rate-limiting, for the formation of volatile Se. In this case, plants produced volatile dimethylselenide (DMeSe) from methionine (Met). The specific route involves methionine being initially methylated by S-adenosyl-L-Met:L-Met S-methyltransferase (MMT) to form Se-methyl Se-methionine (SeMeMet), which is the precursor of the volatile DMeSe typically produced by Se non-hyperaccumulator plant species (Chauhan et al. 2019; Schiavon and Pilon-Smits 2017a). Whether overexpressing SeMeMet can cause an actual increase of Se volatilization has not been verified yet.

In addition to improving the flow of SeCys toward the formation of volatile compounds for averting nonspecific incorporation of Se-amino acids into proteins, plants can break down SeCys into alanine and elemental Se through the activity of selenocysteine lyase (Sec-lyase) (SL) (White et al. 2016). Garifullina et al. (2003) overexpressed a mouse SL in *A. thaliana*, which resulted in the reduced content of Se in proteins and concomitant greater Se accumulation. Interestingly, when the overexpression of SL was targeted to the cytosol, the plants were more tolerant to Se, but when it was targeted to the chloroplast, plants behaved in the opposite way, being quite sensitive to Se. One possible hypothesis is that elemental Se released from SeCys breakdown replaced Fe in Fe-S clusters of electron-transfer proteins functioning in photosynthesis, making them less stable and active (Hallenbeck et al. 2009).

A chloroplastic SL homolog of the mouse SL was identified in *A. thaliana* and named CpNifS. Its overexpression in *A. thaliana* allowed plants to accumulate more Se in their tissues and be more tolerant to it than the wild type. In addition, plants contained less S in proteins, which means a lower rate of Se-amino acid embedding in proteins and a concomitant increase in S concentration (Van Hoewyk et al. 2005). Therefore, the flow of Se was probably diverted to pathways that produce less toxic forms of Se.

The main differences between Se-hyperaccumulators and non-hyperaccumulators in Se and S metabolism are reported in Table 16.2.

Table 16.2 Differences between Se hyperaccumulator and non-hyperaccumulator plant species with respect to Se/S metabolism and main Se forms accumulated

Se hyperaccumulators	Se non-hyperaccumulators
High specificity for Se over S resulting in high Se/S ratio	Low specificity for Se over S resulting in low Se/S ratio
High Se metabolic flow. Unique APS2	Low Se metabolic flow
Additional pathways for Se detoxification	No specific pathways for Se detoxification
Se volatilization as dimethyldiselenide (DMDSe)	Se volatilization as dimethylselenide (DMSe)
High Se-amino acid uptake	Low Se-amino acid uptake
Se mainly accumulated in organic forms. Major organic form of se stored: SeMeCys	Se mainly accumulated in inorganic forms. Major organic form of Se stored: SeMet

16.2.4 Manipulation S-Unrelated Genes for Increasing se Tolerance

In recent years, genetic engineering has targeted other genes, not related to S/Se metabolism but rather to antioxidant defense, for modulating plant Se tolerance and accumulation. For example, overexpression of the selenium-binding protein gene *SBP1* in *A. thaliana* enhanced the resistance of plants to selenite (Agalou et al. 2005) and cadmium (Hugouvieux et al. 2009). SBP1 has many potential metal-binding sites and can chelate selenite, but not selenate, with a ligand to protein molar ratio of 1:1 (Schild et al. 2014). In this Se-SBP1 assemblage, selenite is reduced to form an R-S-Se(II)-S-R-type complex. SBP1 in *A. thaliana* is reported to be associated with cellular S demand as it is upregulated by S starvation and reduces plant sensitivity to multiple stresses through a GSH-dependent mechanism (Zechmann 2020). Furthermore, overexpression of SBP1 can prevent the oxidative stress generated by selenite that may be responsible for mitochondrial damages and dysfunction (Dimkovikj and Van Hoewyk 2014).

Similar to SBP1, the overexpression of the ethylene response factor ERF96 in *A. thaliana* resulted in enhanced tolerance to selenite due to low Se accumulation and increased antioxidant activity (Jiang et al. 2020). In contrast, ERF96-silenced plants were more sensitive to selenite than the wild type. In *A. thaliana*, the ERF96 gene is typically upregulated by selenite, and the mechanism by which it confers greater tolerance to Se is that it reduces the expression of selenite/phosphate transporters PHT1;1 and PHT2;1, thus restricting selenite uptake and allocation in the plant. Furthermore, transgenics overexpressing ERF96 exhibited superior activity of antioxidant enzymes (e.g., catalase, CAT, and glutathione peroxidase, GPX), contained more GSH and decreased ROS accumulation when compared to the wild-type plants. Interestingly, two allelic lines defective in the expression of the gene encoding the cytosolic ascorbate peroxidase (APX1) were Se resistant and accumulated more Se than the wild type (Jiang et al. 2016). Also in this case, Se tolerance was attributed to greater activity of antioxidant enzymes CAT, GPX, and glutathione reductase (GR), and increased synthesis and accumulation of GSH.

The role of GSH in improving Se tolerance by acting as powerful antioxidant was also proved by Bañuelos et al. (2005), who overexpressed GSH synthase in *B. juncea* thus increasing its capacity to tolerate and accumulate Se (Bañuelos et al. 2005), and by Zhou et al. (2018), who reported increased expression of the glutathione S-transferase family gene GST-u4 in leaves of the Se-hyperaccumulator *Cardamine hupingshanensis*, supporting the formation of glutathione-chelated selenate to form Se-binding phytochelatins (PCs) to be transferred into the vacuoles for Se sequestration via ATP-binding cassette transporters (ABCC).

Another fascinating gene unrelated to S/Se metabolism that may be of interest for genetic engineering is the one encoding a broccoli methyltransferase (*BoCOQ5–2*) involved in the ubiquinone biosynthetic pathway and reported to stimulate selenium volatilization in both bacteria and transgenic *A. thaliana* plants (Zhou et al. 2009, 2010). Ubiquinone has antioxidant functions, and thus it would act in cells by protecting them and more specifically mitochondria from oxidative stress generated by excess Se (Bentinger et al. 2007). When BoCOQ5–2 was expressed in *A. thaliana*, plants volatilized three times more Se in the form of DMDSe than the wild type and became more tolerant to Se (Zhou et al. 2009). The increase in tolerance was primarily attributed to the restriction of ROS generation rather than to a direct effect of manipulation of the ubiquinone pathway.

Additional genes of interest have been discovered in *A. thaliana* (Van Hoewyk et al. 2008), *S. pinnata* (Wang et al. 2018), and *C. hupingshanensis* (Zhou et al. 2018) with key roles in Se detoxification pathways and that may alter Se metabolism. More specifically, the genes of interest are those involved in the transamination of SeCys or its oxides (e.g., L-cysteate and L-cysteine-sulfinate), selenoprotein degradation, synthesis and signaling of ethylene and abscisic acid, glutamyl cycle by recycling glutamate from GSH-conjugates (e.g., glutamyl cyclotransferase (GGCT2;1), and selenation reactions (e.g., genes coding for aryl sulfotransferases). In the last case, the flow of selenate in the S pathway was found to be diverted to form phosphoadenosine 5′-phosphoselenate (PAPSe) and used for selenation in the root of the Se hyperaccumulator *C. hupingshanensis*; thus, selenide, SeCys, and selenoprotein formation was prevented when plants were subjected to high Se doses (Zhou et al. 2018).

With respect to genes that control Se-protein degradation, in the study by Zhou et al. (2018), the genes coding for E3 ubiquitin-protein ligase MUL1, RNF13, and the ubiquitin- conjugating enzyme E2 7 were upregulated by Se in the roots, with gene expression levels similar to those reported for *Stanleya pinnata* (Sabbagh and Van Hoewyk 2012). Therefore, expressing these genes in Se non-hyperaccumulators might be relevant for reducing the toxicity stemmed from the generation of malformed proteins under Se stress.

16.3 Potential of Genetic Engineering for Se Biofortification and Phytoremediation

Studies conducted so far indicate that several S-related and unrelated genes could be valid candidates for manipulating plant tolerance and accumulation of Se in crops to be fortified or in high biomass plants for use in phytoremediation of Se-polluted soils (Fig. 16.1) (Zhu et al. 2009; Pilon-Smits and LeDuc 2009; Schiavon and Pilon-Smits 2017b).

Even though high Se levels in harvestable plant parts are desirable for both biofortification and phytoremediation, some specific traits appear to be more suitable than others depending on the phytotechnology applied. For example, in the case of biofortification, we aim at increasing the accumulation of Se in edible plant organs mainly in the forms most available to humans and most beneficial to health. Therefore, genes whose overexpression might favor the entry of Se into the plants and its flow along the S assimilation pathway to produce elevated amounts of SeMet and MetSeCys are the main targets to be addressed by genetic engineering. As for phytoremediation, genes promoting Se accumulation, root-to-shoot translocation, and further volatilization into the atmosphere are the most attractive to be overexpressed. In both phytotechnologies, however, increasing the plant tolerance to Se is mandatory to avoid an unintended reduction of plant growth and yields, which could otherwise hinder the final outcome of plant enrichment with Se.

It should be noted that, despite the promising results obtained in laboratory and greenhouse tests, only a few field testing have been performed to assay the capacity

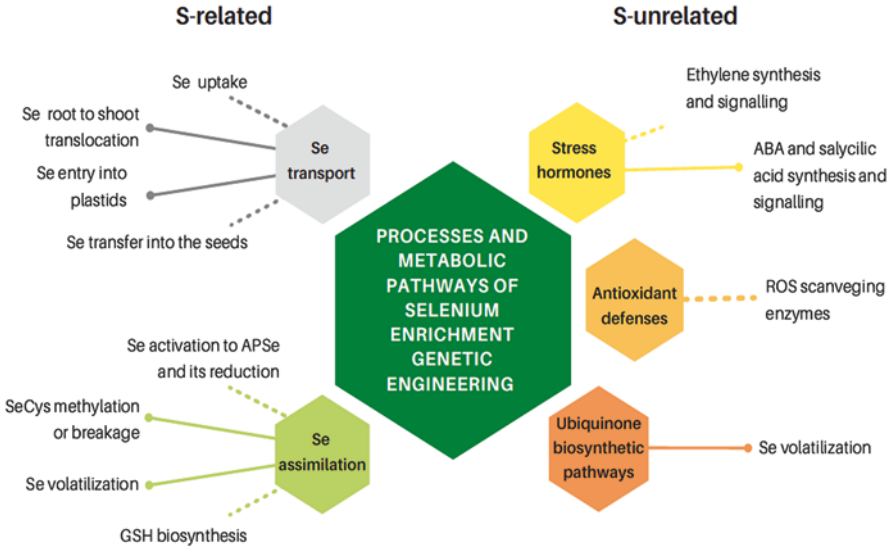

Fig. 16.1 Survey of principal S-related and unrelated processes/pathways targeted by genetic engineering for plant Se enrichment

of transgenics to accumulate and tolerate Se or remediate Se-contaminated sites by increasing Se volatilization rates. This is mainly to the fact that in many countries, the cultivation of transgenics is not allowed, and their use in biofortification and phytoremediation technologies poses some concerns and is less accepted by the populations than agronomic biofortification and conventional breeding (Zhu et al. 2009).

Transgenic lines of *B. juncea* overexpressing either chloroplastic SL or SMT manifested increased capacity for Se phytoremediation when grown under field conditions (Bañuelos et al. 2007). They could indeed accumulate up twofold more Se in their shoot from Se-contaminated saline sediments and produced more biomass than wild-type plants.

Further small-scale experiments testing transgenics used *B. juncea* lines overexpressing genes encoding the enzymes APS, γ-glutamyl-cysteine synthetase (ECS), and glutathione synthetase (GS) (Bañuelos et al., 2005). The transgenic plants showed greater biomass yield and, in the case of APS transgenics, up to 4.5-fold more Se accumulation than wild-type plants.

These studies on the whole support the evidence that genetic engineering can feasibly generate plants that are really effective for Se phytoremediation purposes, as well as crops fortified with Se. In particular, wide transcriptomic studies conducted so far in Se-hyperaccumulators and accumulators have provided a pool of genes from which to draw to create transgenics with altered Se accumulation or metabolism (e.g., Çakir et al. 2015; Van Hoewyk et al. 2008; Wang et al. 2018; Zhou et al. 2018). Studies from Çakir et al. (2016) and Huang et al. (2010) additionally suggest a potential role of several miRNAs (i.e., small RNA molecules controlling targeted gene translation) in the modulation of Se metabolism which deserve a deeper investigation (Fig. 16.2).

With respect to Se biofortification, a feasible avenue for improving enrichment of plants with Se could include attempts to overexpress targeted gene(s) in specific plant tissues and organs and produce anticarcinogenic compounds (e.g., MetSeCys) that can be extracted in appreciable quantities. In the case of phytoremediation, genes whose overexpression could increase Se tolerance and accumulation and especially promote Se volatilization into nontoxic forms appear the best candidate for the genetic engineering of plants ideally suited for the cleanup of Se-contaminated soils.

16.4 Considerations and Future Directions

A number of genes and metabolic pathways have been identified as potential targets of Se genetic engineering for the generation of transgenics with superior capacity of Se enrichment. However, although a promising tool, the use of transgenics in phytoremediation and biofortification is still largely limited and far from being accepted by local populations in several countries. To overcome this limitation, new molecular tools like CRISPR/Cas9 could be used to modify the genetic code of the plants

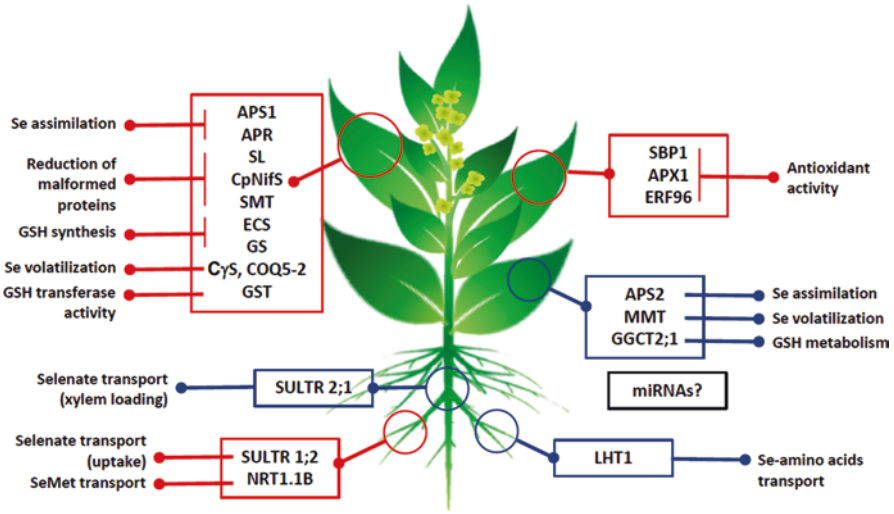

Fig. 16.2 Survey of genes overexpressed in plants and conferring increased Se tolerance and accumulation (reported inside red squares) and promising genes yet to be tested (reported inside blue squares). APX1 is the only among these genes whose loss of function was found to be associated with enhanced Se tolerance and accumulation. For all genes, the metabolic target or the process in which they are implied is indicated on the side. The role of miRNAs in the control of Se metabolism is unknown and might deserve investigation

without introducing foreign DNA, with the aim of generating plants that possess transporters (e.g., SULTR) or enzymes (e.g., APS, APR, etc.) with constitutive expression or high specificity for Se. In this case, the technology would benefit from the high-throughput sequencing of hyperaccumulator genomes to generate plants with greater Se storage capacity and tolerance that may gain better public acceptance.

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