# Chapter 10
# Biochemical and Molecular Aspects in Phytoremediation of Selenium

**L.F. De Filippis**

**Abstract** The element selenium (Se) is considered a finite and non-renewable resource on earth, and has been found to be an essential element in humans, animals, micro-organisms and some other eukaryotes; but as yet its essentiality to plants is in dispute. There is no doubt that adequate levels of selenium are important to animal and human health, and some selenium compounds have been found to be active against cancers. A limited number of plants growing on selenium rich soils can accumulate very high levels of selenium (i.e., hyperaccumulate selenium), and are classified as selenium tolerant, however, many more plants do not accumulate selenium to any great extent, and are selenium sensitive. Plants vary considerably in their physiological and biochemical response to selenium, and a revision of the physiological responses of plants to selenium is presented; especially growth, uptake, transport and interaction of selenium with other minerals. The review also details the biochemical responses of plants to selenium, the assimilation of selenium in plants and possible incorporation into proteins. Molecular approaches to understanding selenium toxicity and tolerance have increased the knowledge of mechanisms of action, and the molecular biology of selenium in transgenic plants is detailed; with special reference to the similarity with sulphur metabolism, sulphur/selenium transporters and important assimilation enzymes. Phytovolatilisation of selenium will be summarised, which is a unique method for plants to metabolise selenium to more volatile forms in order to eliminate selenium from tissues, and eventually from the soil and water. Finally, the application of phytoremediation in selenium rich environments is reviewed in light of the possible use of plants to decontaminate selenium from soil and water environments, and perhaps also produce a product which could be used in mineral supplementation of foods, and even fighting cancers.

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**Abbreviations**

| | |
|---|---|
| APS | adenosine 5'-phosphosulphate |
| APSe | adenosine 5'-phosphoselenate |
| Cys | cysteine |
| Cysth | cystathione |
| DMS | dimethylsulphide |
| DMSP | dimethylproprionate |
| DMSe | dimethylselenide |
| DMDSe | dimethyldiselenide |
| DMSeP | dimethylselenioproprionate |
| GPX | glutathione peroxidase |
| GSH | glutathione |
| GSSeSG | selenodiglutathione |
| HAST | high affinity sulphate transporter |
| LAST | low affinity sulphate transporter |
| MeCys | *S*-methylcysteine |
| MeSeCys | *S*-methylselenocysteine |
| MeSeCysSeO | methylselenocysteine seleno-oxide |
| Met | methionine |
| S | sulphur |
| Se | selenium |
| SeCys | selenocysteine |
| Secysth | selenocystathione |
| SeGSH | selenoglutathione |
| Sehocys | selenohomocysteine |
| SEM | SeCys + MeSeCys |
| SeMet | selenomethionine |
| SeMMet | selenomethylmethionine |

## Contents

## 1 Introduction

The element selenium (Se) was discovered in 1817 by the Swedish chemist Berzelius, Jons Jakob and named after the Greek moon goddess ‘selene’. Selenium belongs to the Periodic Table Group VIA; the group that also contains sulphur (S) and tellurium (Te). However Se compounds, minerals and seleniferous soils have a long history. In 1295 Marco Polo reported that during his famous journey from Venice through Asia Minor to China, his horses suffered from a typical necrotic hoof disease when the horses ate poisonous plants; the symptoms are now known to be due to Se toxicity from animals ingesting high levels of Se present in accumulator plants (Birringer et al. 2002). As early as 1842 evidence became available for the toxicity of Se to animals, but the first recorded written evidence of Se poisoning in livestock was reported in 1856 by the US Army surgeon, Madison (Whanger 2002). In 1884 a television system was developed using Se pohotocell technology in imaging (Chasteen and Bentley 2002). Therefore Se played a fundamental role in xerography, or in other words early versions of televisions and photocopiers. The photoconductivity of Se compounds has had a profound influence on humanity, and Se compounds have found many roles in the electrical, electronic and semiconductor industries. As well, Se is often used in agriculture, paint and pigment production, volcanisation, oil refinery, glass manufacturing, coal and electricity generation, metallurgy and lately medicine (Lemly 1997; 2004).

The toxicity of Se and Se compounds in domestic animals had been identified and described for many years, however it was not until the Kesterson Reservoir

controversy in the USA in the 1980s that scientists and health regulators were made aware of Se as an environmental contaminant. The reservoir contamination was traced back to Se loaded agricultural drainage water, which had been allowed to flow into the reservoir from adjoining farms (Ohlendorf et al. 1986; Saiki and Lowe 1987). Interest in the environmental impact of Se has increased since this incident 25 years ago. However in nature, Se toxicity is more often found in arid and semi-arid regions of the world that have seleniferous, alkaline soils derived from weathering of Se rich rocks and shales. Contamination of land and water by Se is inevitable due to the geochemical balance of sulphur versus selenium (i.e., ratio of S:Se) being roughly 3000:1 in rocks while the same balance in waters are closer to 8,000,000:1. Seleniferous soils exist in China's Great plains, Canada, a belt in Mexico, pockets in Latin America, parts of New Zealand and Australia, North-West and Great plains regions of the USA, parts of Ireland, in Russia and the Punjab in India (Baker and Brooks 1989; Baker et al. 2000; Dhillon and Dhillon 2003; Haug et al. 2007; Sharma et al. 2009).

In trace amounts, Se is an essential micronutrient and has important beneficial roles in microorganisms, animals, a number of other eukaryotes and humans. However Se has not been shown to be an essential microelement to vascular plants (Pilon-Smits et al. 2009). Nutrition and health benefits of Se include combating heart disease, thyroid disease (hypothyroidism) and strengthening the immune system (Hartikainan 2005). Numerous studies have also demonstrated the anti-carcinogenic role that some organic forms of Se have, especially lung, colon and prostrate cancer, with the most responsive cancers being prostrate and lung cancers (Ellis and Salt 2003; El-Bayoumy and Sinha 2005). It is also true that Se and Se resources could be described as non-renewable and in many cases compounds in short supply around the world, and there is a strong case not only to protect Se resources and minerals, but also to find better ways of extracting Se resources for nutritional and health reasons. Haug et al. (2007) have provided a world Se budget which clearly demonstrates how vulnerable and in short supply Se is around the world, and we should begin to address this problem and how we use this scarce resource.

Environmental pollution of Se can have an impact on human health, agricultural productivity and the stability of natural ecosystems. Even low-level contamination if present on a large enough scale can represent large economic and logistical barriers to effective and timely treatment. At present, aggressive engineering based technologies and/or excavation and entombment of Se contaminated sites may not be cost effective, and therefore not easily justified; and at any rate it may have marginal impacts (Berken et al. 2002; Rugh 2004). Therefore *in situ* biological remediation could be the most appropriate corrective option for treatment of a wide range of low impact contamination due to Se. In many situations, and because of the low toxicity of Se contamination the economic value placed on remediating this type of pollution is often not considered a high priority. However if decontamination is coupled to an economic positive outcome from the extracted material, as could be achieved in the case of Se, then the economics could well be different; especially if a Se rich bi-product could be manufactured for a world-wide scarce resource. Bioremediation typically refers to microbial mediated processes which attempt to clean a site, while

phytoremediation refers to plant mediated clean-up procedures. Part of this review will deal with biological aspects of phytoremediation of Se contaminated areas, but for a more general review of phytoremediation see Pilon-Smits (2005) and Banuelos (2006).

The chemistry of Se has been reviewed extensively by a number of authors (Birringer et al. 2002; Pilon-Smits et al. 2009) and this review will only basically cover areas of need. Se important in human health and cancer treatment has also been well reviewed recently (Combs 2005; El-Bayoumy and Sinha 2005), and we will not deal with these topics. Excellent reviews on Se in higher plants were published by Terry et al. (2000) and Sors et al. (2005b), and we intend to concentrate on more recent developments, and focus on bioremediation implications, although the physiology, biochemistry and molecular biology of Se must at times refer back to these reviews.

## 2 Physiology

### *2.1 Types of Se Accumulator Plants*

Most plants contain naturally low tissue concentrations of Se, typically less than 5 mg Se $kg^{-1}$ dry weight; and rarely does Se content exceed 15 mg Se $kg^{-1}$ dry weight in plants. This is true even if plants have been grown in high Se containing soils, although compared to controls in soils low in Se they do take-up more Se; these plants are called Se non-accumulators (Ernst 1982; Baker and Brooks 1989; Mayland et al. 1989; Bell et al. 1992). A limited number of plants, especially from the Fabaceae and Brassicaceae can accumulate considerably higher levels of Se in leaves, and are often found on soils that are naturally enriched with Se (i.e., seleniferous soils). These accumulator plants can be further sub-divided into two groups (Dhillon and Dhillon 2003; White et al. 2007):

**(a) Primary accumulators (hyperaccumulators)** – which have concentrations of Se in leaves in the range of 70–300 mg Se $kg^{-1}$ dry weight, and discrimination coefficients (DC) between Se and S (Se/S) of more than 2.5 in solution culture. DC = [Se/S] plant / [Se/S] solution. Examples include various species of *Astragalus, Stanleya pinnata, Melilotus officinalis, Grindelia squarrosa, Neptunia amplexicaulis, Bertholletia excelsa*, and species of *Lecythis, Morinda, Happlopappus* and *Machaerantha* (Marschner 1995, White et al. 2004).

**(b) Secondary accumulators** – which take-up Se in proportion to the amount of Se available in the soil and have a DC of less than 2.5. Tissue concentrations of Se are in the range of 5–30 mg Se $kg^{-1}$ dry weight. Plants in this group include species of *Aster*, *Attriplex, Brassica juncea* and *Brassica napus* (canola), species of *Comondra, Grayia, Gutierrezia, Siderenthus* and *Castileja* (Huang and Wu 1991; White et al. 2004).

A list of tested primary and secondary accumulator plant species is given in Table 10.1, although only about 185 plant species were tested by White et al. (2004)

**Table 10.1** Plant family and species grown in hydroponic solution containing 1 m*M* sulphate and 0.5 μM selenate with leaf Se concentrations and DC (Se/S ratio) values of greater than 1.4 shown. Species with DC values of less than 1.4 are not shown, and the list is modified from data compiled by White et al. (2004, 2007)

| Family | Species | Leaf Se (mg $kg^{-1}$ dw) | DC (Se / S) |
|---|---|---|---|
| Fabaceae | *Astragalus racemosus* | 282.8 | 14.14 |
| | *Trifolium subterraneum* | 14.6 | 1.76 |
| | *Astragalus sinicus* | 6.9 | 1.68 |
| | *Medicago lupina* | 5.7 | 1.64 |
| | *Trifolium repens* | 7.5 | 1.62 |
| | *Trifolium pratense* | 6.4 | 1.53 |
| | *Medicago sativa* | 6.0 | 1.41 |
| Brassicaceae | *Stanleya pinnata* | 68.6 | 3.27 |
| | *Brassica nigra* | 17.9 | 2.37 |
| | *Raphanus sativa* | 22.2 | 1.93 |
| | *Brassica arvense* | 24.4 | 1.75 |
| | *Brassica carinata* | 17.0 | 1.72 |
| | *Sinapis alba* | 21.9 | 1.70 |
| | *Brassica juncea* | 21.0 | 1.63 |
| | *Brassica oleracea* | 33.0 | 1.51 |
| Solanaceae | *Solanum tuberosum* | 9.8 | 2.02 |
| | *Solanum melongena* | 5.8 | 1.80 |
| | *Lycopersicon pennellii* | 21.2 | 1.77 |
| Poaceae | *Panicum miliaceum* | 11.9 | 2.21 |
| | *Oryza sativa* | 11.3 | 2.12 |
| | *Cynodon dactylon* | 14.1 | 1.97 |
| | *Bromopsis inermis* | 12.7 | 1.91 |
| | *Agrostis stolonifera* | 13.8 | 1.90 |
| | *Boutelouga gracilis* | 6.9 | 1.87 |
| | *Dactylis glomerata* | 7.1 | 1.80 |
| | *Hordeum vulgare* | 12.3 | 1.73 |
| | *Holcus lanatus* | 8.7 | 1.70 |
| | *Sorghum bicolor* | 7.1 | 1.67 |
| | *Lolium multiflorum* | 7.5 | 1.65 |
| | *Sporobolus airoides* | 8.8 | 1.60 |
| Asteraceae | *Machaeranthera tanacetifolia* | 15.2 | 1.81 |
| | *Helianthus annuus* | 7.6 | 1.61 |
| | *Machaeranthera bigelovii* | 5.7 | 1.51 |
| Caryophyllaceae | *Atriplex hortensis* | 6.5 | 1.41 |
| | *Beta vulgaris* | 5.6 | 1.36 |
| Malpighiaceae | *Linum usitatissimum* | 13.4 | 1.90 |
| Cucurbitaceae | *Cucumis sativa* | 10.4 | 1.62 |

and White et al. (2007). It is worth noting that although there is a relationship between higher Se accumulation and a higher DC ratio, this is not always true. For example, *B. arvense, B. juncea* and *B. oleracea* have moderate DC ratios of 1.50–1.75 yet contain high leaf Se content (21–33 mg Se $kg^{-1}$ dry weight), but in contrast *B. gracilis, D. glomerata* and *S. melongena* have high DC ratios of 1.80–1.87 yet contain low leaf Se content (5.8–7.1 mg Se $kg^{-1}$ dry weight). Se accumulators certainly can grow on seleniferous soils, but not all plant species growing there may accumulate Se. For example, the genera *Astragalus* contains both Se accumulator species and Se non-accumulator species, and these different types of plants can grow next to one another on the same soil. Most forage and crop plants, as well as grasses contain less than 5 mg Se $kg^{-1}$ dry weight in their tissues, and therefore are classified as non-accumulators (Ernst 1982; Baker et al. 2000; Freeman et al. 2006).

Chemical forms of Se accumulated in crops and other important dietary products to humans are summarised in Table 10.2. It is apparent from this table that most crop plants accumulate Se as SEM (SeCys + SeMCys), and the problem with this is not so much the chemical form of Se found, but that levels in most of these crop plants is too low for dietary needs. On the other hand phytoplankton mostly have a very low Se concentration and Se is mostly as selenite. Fish, dairy products, meat and milk have Se mostly in the form of selenate and selenite, and this is also not satisfactory. Fortified crop plants tested so far accumulate Se mainly in the form of SeMCys, but wether this is the desired chemical form or not required for human nutrition has not been thoroughly tested. It is assumed from very few reports on experimental animals like the rat values in Table 10.2, that the chemical form of Se important in human diets and even cancer prevention is an organic form of Se (El-Bayoumy and Sinha 2005; Haug et al. 2007; White and Broadley 2009). The conclusion from Table 10.2 is that young sprouting seedlings of fortified crops best achieves the beneficial and dietary needs for humans.

## *2.2 Se Toxicity and Tolerance*

When Se sensitive plants are exposed to elevated levels of Se in the soil root medium they may exhibit varying symptoms such as stunted growth, chlorosis, withering, drying of leaves and premature death of the whole plant (Mengel and Kirkby 1987; Mikkelson et al. 1989). There are differences between Se accumulator and Se non-accumulator plants in the threshold values of Se that determine toxicity:

**(a) Primary accumulator plants** – Se toxicity is shown at values between 2000 and 4000 mg Se $kg^{-1}$ dry weight shoots. Plants in this group include *Astragalus, Stanleya, Neptunia* and *Brassica* (Broyer et al. 1972; Galeas et al. 2007).

**(b) Secondary accumulator plants** – Se toxicity shown at values between 75 and 900 mg Se $kg^{-1}$ dry weight shoots. Plants tested in this group include clover, strawberry clover, bent grass, ryegrass, rice, buffalo grass, alfalfa and tall fescue (Wu et al. 1988; Sharma et al. 2009).

**(c) Non-accumulator plants** – Se toxicity shown at values between 2 and 25 mg Se $kg^{-1}$ dry weight shoots. Plants tested in this group include wheat, rice, pea, mustard, kidney beans and alfalfa (Zayed et al. 1998; Sharmasarkar and Vance 2002).

**Table 10.2** Distribution and percentage of different selenocompounds identified in various biological and food materials. Modified from Whanger (2002) and Hartikainan (2005). SEM is the sum of SeCys plus MeSeCys

| Plant species (type) | Selenate | Selenite | SEM | SeCys | MeSeCys | Others |
|---|---|---|---|---|---|---|
| Wheat grains | 12–19 | | 56–83 | 24–32 | 11–24 | 4–26 |
| Wheat straw | 97 | | | | | 3 |
| Corn | | | 61–64 | 15–16 | | 20–24 |
| Rice | 1–3 | 5–13 | 68–81 | 6–10 | | 19–31 |
| Soybean | | | >80 | | | |
| Lucerne | 5–5 | | 70–81 | | | |
| Ryegrass | 10–15 | | 66–78 | | | |
| Red clover | 5–8 | | 72–81 | | | |
| Grassland legumes | | | 51–70 | 19–39 | 10–13 | |
| Vegetables (20 types) | 1–50 | | 40–50 | | | |
| Lettuce | 10–12 | | 35–40 | | | |
| Tomato | 15–20 | | 55–65 | | | |
| Oil seeds and nuts | 10 | 25 | 40 | 15 | 25 | |
| Phytoplankton | 1 | 83 | 3.2 | | 12.8 | |
| *Astragalus prelongus* | 1.4 | 9 | 37 | | 52 | |
| *Arabidopsis thaliana* | 25 | 15 | 40 | 5 | 10 | |
| Rats (selenite injected) | | | 16–30 | 24–40 | | 20–34 |
| Rats (SEM injected) | | | 14–23 | 22–57 | | 15–40 |
| Enriched yeast | 0–4 | 0–27 | 23–59 | 0–21 | 6–20 | 5–51 |
| Enriched garlic | 2–5 | 8 | | 1–13 | 47–87 | 4–36 |
| Enriched onion | | | | 7–38 | 42–55 | 21–35 |
| Enriched broccoli (sprouts) | 10 | | 25 | 30 | 25 | 15 |
| Enriched broccoli (florets) | 5 | | 25 | 21 | 23 | 21 |
| Enriched leeks (bulbs) | 12–25 | | | | 35–50 | 1–3 |
| Enriched potatoe | | | | 15–20 | 50–60 | 5–10 |
| Fish (17 different types) | 15–36 | 5–30 | | | | |
| Dairy products, milk (low and normal fat) and eggs | 5.4 | 25 | 30 | | | |
| Meat products | 10–20 | 25–50 | 10–20 | | | |
| Commercial Se feed supplement for livestock | 0.6 | 98.7 | 0.7 | | | |

The threshold range in non-accumulator plants generally vary with plant age and sulphur supply. Younger plants can be more susceptible to toxicity, and tolerance to Se toxicity increases with increasing sulphate supply (Brown and Shrift 1981). The threshold toxic value in non-accumulator plants also depends on the form of Se applied; with selenate and selenite being the main toxic forms to plants. This may be linked to both these forms of Se being readily absorbed and translocated in plants and assimilated in the inorganic forms (Eustice et al. 1980). In most studies selenate is more toxic to plants than selenite (Sors et al. 2005b).

The predominant mechanism involved in Se toxicity is almost certainly due to the incorporation of SeCys and SeMet into proteins in place of Cys and Met (Anderson and Scarf 1983). Additionally, Se may diminish the actual rate and efficiency of

protein synthesis because the substitution of Se amino acids into proteins may mean a less effective or slower rate of protein synthesis during translation (Eustice et al. 1981). But there may be other mechanisms involved such as effects on chlorophyll biosynthesis, as demonstrated by the symptoms of chlorosis. Interference with the reduction of nitrate in leaves and the inhibition of glutathione accumulation are other possible effects. Glutathione levels are critical in anti-oxidative reactions and stress, and evidence suggests Se decrease these reactions, but may also diminish plant defence mechanisms against disease organisms (Aslam et al. 1990; Mugesh et al. 2002; Sharma et al. 2007). It is worth noting however that high levels of Se, especially in hyperaccumulating plants have been shown to protect the plant from leaf chewing insects and other herbivorous animals eating the plants (Boyd 2007; Freeman et al. 2007; Freeman et al. 2009).

A number of possible modes of tolerance to toxic compounds have been described by Pilon-Smits (2005) and may involve any of six mechanisms; these include differences in adsorption, conjugation, sequestration, enzymatic modification, enzymatic degradation and volatilisation. Tolerance in Se accumulator plants appears to be due to a number of mechanisms under the categories above (Neuhierl et al. 1999; Wang et al. 1999; Ellis and Salt 2003):

(a) Decrease in excessively high concentrations of Se being transported into cells of leaves (adsorption/transportation).
(b) Accumulation of Se in Se amino acids, but these seleno-amino acids are not incorporated into normal protein synthesis (sequestration)(enzymatic modification).
(c) Compartmentation of Se as selenate in the vacuole and away from more sensitive cytoplasmic reactions (sequestration).
(d) Increase ATP sulphurylase and SeCys methyltransferase activities to reduce inorganic Se to organic forms of Se, although other enzymes and reactions are also required (enzymatic modification).
(e) Conjugation with glutathione (GSH) and an increase in anti-oxidation protective reactions (conjugation).
(f) Conjugation with Se binding proteins and polypeptides, decreasing inorganic Se content (conjugation).
(g) Increase volatilisation of mainly organic forms of Se out of plant cells and tissues (volatilisation).

In tolerance mechanisms, the key role of the two enzymes ATP sulphurylase and SeCys methyltransferase are of prime importance, and these enzymes have been the main focus of more recent studies in Se tolerance, including transformation and use of transgenic plants with increased tolerance to Se. However, recently the role of Se specific and non-specific binding proteins and polypeptides are being increasingly recognised as having additional effects in increasing Se tolerance (see Table 10.3 for a summary).

**Table 10.3** Summary of molecular genetic studies and transgenic reports on plants associated with effects on selenium tolerance and selenium accumulation, including the origin of the genes

| Transgene | Gene origin (plant species) | Transgenic plant species | Effects on Se tolerance and accumulation | Reference |
|---|---|---|---|---|
| *SMT* Selenocysteine methyltransferase | *A. thaliana* | *B. oleracea* | Upregulation caused higher Se levels, but little effect on Se toxicity; complex interactions with S | Lyi et al. (1995) |
| *APS2* isoform of ATP sulphurylase | *A. thaliana* | *N. tabacum* | No significant effects on Se accumulation and Se tolerance | Hatzfeld et al. (1998) |
| *APS1* isoform of ATP sulphurylase | *A. thaliana* | *B. juncea* | Increase in Se accumulation and an increase in Se tolerance | Pilon-Smits et al. (1999) |
| *CyS* crystathionine -γ-synthase | *A. thaliana* | *B. juncea* | Lower Se levels in shoots and increased Se tolerance | Van Huysen et al. (2003) |
| *SMT* selenocysteine methyltransferase | *A. bisulcatus* | *A. thaliana* | Increase in foliar Se levels and increase in tolerance to selenite, but not selenate | Ellis et al. (2004) |
| *SMT* selenocysteine methyltransferase | *A. bisulcatus* | *A. thaliana* | Increase in total Se levels and increase in tolerance to selenite, but not selenate | Le Duc et al. (2004) |
| *SMT* selenocysteine methyltransferase | *A. bisulcatus* | *B. juncea* | Increase in total Se levels and increase in tolerance to selenite, but not selenate | LeDuc et al. (2004) |
| *APS* isoform of ATP sulphurylase | *A. thaliana* | *B. juncea* | Increase in Se accumulation and an increase in Se tolerance | Van Huysen et al. (2004) |
| *CyS* Crystathionine -γ-synthase | *A. thaliana* | *B. juncea* | Lower Se levels in shoots and increased Se tolerance | Van Huysen et al. (2004) |

**Table 10.3** (continued)

| Transgene | Gene origin (plant species) | Transgenic plant species | Effects on Se tolerance and accumulation | Reference |
|---|---|---|---|---|
| *APS1* isoform of ATP sulphurylase | *A. thaliana* | *A. thaliana* | Decreased Se accumulation and Se tolerance | Sors et al. (2005) |
| *PaAPR* APS reductase | *A. thaliana* | *A. thaliana* | Decrease in foliar Se and increase selenate tolerance | Sors et al. (2005) |
| *SATm* Mitochondria serine acetytransferase | *T. goesingense* | *A. thaliana* | No significant effects on Se accumulation and tolerance | Sors et al. (2005) |
| Selenium binding polypeptides/proteins (SBP) | *A. thaliana* | *A. thaliana* | Resistance to Se achieved due to overexpression of Se binding proteins | Agalou et al. (2005) |
| *AtCpNifS* chloroplast protein like Se Cys lyase | *A. thaliana* | *A. thaliana* | Enhanced selenate tolerance by reducing Se incorporation into protein | Van Hoewyk et al. (2005) |
| A number of Se associated genes and gene families | *A.thaliana* | *A. thaliana (RT-PCR used to detect upregulation)* | Se tolerance was linked to upregulation (higher) activity of *APS*, *SULTR* and *SMT* | Zhang et al. (2006) |
| Selenocysteine lyase (SeCys lyase) | *A. thaliana* | *B. juncea* | Higher selenate tolerance probably by reducing Se incorporation into protein | Banuelos et al. (2007) |

**Table 10.3** (continued)

| Transgene | Gene origin (plant species) | Transgenic plant species | Effects on Se tolerance and accumulation | Reference |
|---|---|---|---|---|
| *SMT* Selenocysteine methyltransferase | *A. thaliana* | *B. juncea* | Increase in total Se levels and increase in tolerance to selenite, but not selenate | Banuelos et al. (2007) |
| *SULTR 1,2,3* Sulphate proton transporters | *A. thaliana* | *A. thaliana (knock-down gene technology)* | Selenate accumulation reduced by HAST transport, little effect on selenite | Lydiate et al. (2007) |
| *AtCpNifS* chloroplast protein like Se Cys lyase | *A. thaliana* | *A. thaliana* | Confirm higher selenate tolerance by reducing Se incorporation into protein | Van Hoewyk et al. (2008) |
| *SBP 1,2,3* Se binding protein gene family | *A. thaliana* | *A. thaliana* | Elevated tolerance to heavy metal cadmium (Cd) by Se protein also binding Cd | Dutilleul et al. (2008) |

## 2.3 Se Uptake and Transport

Selenate is accumulated in plant cells against an electrochemical potential (or gradient) by active transport driven by ATP (ATPase). Selenate readily competes with the uptake of sulphate, and both anions appear to be taken-up by a number of sulphate transporters in the root plasma membrane (Abrams et al. 1990). The sulphate transporters modulate Se uptake in bacteria and yeasts, and at least two types of these transporters are also present in plants. The S/Se transporters described belong to two main classes (Fig. 10.1):

**(a) Transporters that have high affinity for sulphate (HAST)**. This is likely to be the primary transporter involved in sulphate uptake from the soil, and is expressed mainly in roots with a $K_m$ for sulphate of 7–10 μM. HAST is also considered to be involved in selenate uptake; and

**(b) Transporters with a low affinity for sulphate (LAST)**. This secondary transporter is more likely to be involved in intercellular transport of sulphate, expressed in both the roots and shoots with a $K_m$ for sulphate of 100 μM. LAST is also considered to be involved in selenate uptake (Clarkson and Luttge 1991; Smith et al. 1995; Cherest et al. 1997).

Selenite uptake on the other hand may not be mediated by membrane transporters, as hydroxylamine a respiratory inhibitor inhibits selenite uptake by only about 20%, however hydroxylamine inhibited selenate uptake by 80% (Arvy 1997). Abrams et al. (1990) showed that SeMet uptake by wheat seedlings was coupled to metabolism as evident by the inhibition of uptake by the metabolic inhibitor

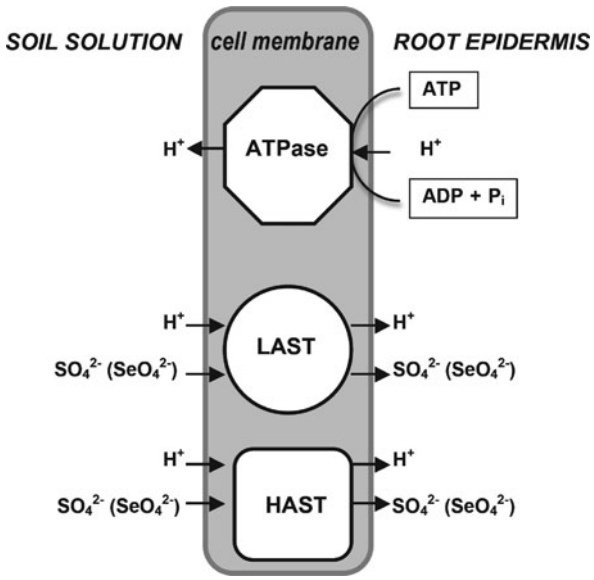


**Fig. 10.1** Selenate and sulphate uptake across the root cell membrane driven by ATP (ATPase). LAST is the low affinity sulphate transporter and HAST is the high affinity sulphate transporter (Modified from Sors et al. 2005b)

dinitrophenol and anaerobic conditions. Se concentrations in xylem exudate in roots exceeded that in the external medium by 6–13 times when selenate was added. However when selenite was added Se concentrations in the xylem were always lower than the outside solution, and tends to confirm that membrane transporters may not be involved in selenite uptake (Smith et al. 1997).

Translocation of Se from the roots to the shoots is highly dependent on the form of Se supplied. Selenate is transported more readily than selenite or organic Se compounds. For example, more than 50% of Se was transported from the roots to the shoots within 3 hours when selenate was added. Whilst less than 10% Se was transported from the roots to the shoots when selenite or organic Se was added (Shrift and Ulrich 1976). The reason may be that selenite is more easily converted to organic Se than selenate, and selenate is more strongly retained in the roots after transportation from the soil to the root by HAST. As well, the other conclusion could be that only selenate is readily available in the roots for transportation to the leaves by LAST. The distribution of Se in plants also differs with the type of Se accumulating plant species under investigation:

**(a) Se accumulators** – Se is accumulated most in young leaves, early vegetative growth, during reproductive stages and seeds; while Se content in mature leaves is reduced greatly (Broyer et al. 1972; Sors et al. 2005a).

**(b) Se non-accumulators** – Se is often similar in seeds and grains, and in the roots; with lower amounts in the stem and leaves (Arvy 1997; Asher et al. 1977).

Apart from the form and concentration of Se being important, the concentration of sulphur present is important (see Sect. 2.4 below). Plants can also absorb volatile forms of Se from the atmosphere, via the leaf surface and stomata. The Se can quickly be translocated down, probably in the phloem and accumulates in the roots as inorganic selenite, selenoglutathione (SeGSH) and protein bound seleno-methionione (SeMet) (Terry et al. 2000).

## *2.4 Se Interaction with Other Salts*

Sulphates compete with selenate for uptake. Sulphate salinity (i.e., $Na_2SO_4$) therefore drastically inhibits plant selenate uptake. However, not all Se type plant species are affected in the same way:

**(a) Se accumulator plants** – selenate is preferentially taken up over sulphate, and so plants can take up high amounts of Se despite the high sulphate salinity present; and

**(b) Se non-accumulator plants** – have high discrimination for sulphate, and selenate uptake can be significantly inhibited by increasing sulphate supply (Banuelos et al. 1995; Zayed et al. 1998).

On the other hand, chloride salinity (i.e., NaCl) has a much reduced effect on Se uptake, but generally there can be a small decrease in shoot accumulation of Se with

increasing NaCl levels (Wu and Huang 1991; Bell et al. 1992); but this may well be more of an indirect effect of NaCl generally decreasing plant metabolism.

Se is often associated with minerals also containing heavy metals, especially Cu, Ag, Hg and U (Broadley et al. 2007) therefore it is not surprising to find interactions between Se and heavy metals. For example De Filippis (1979) demonstrated that selenite and cysteine decreased the sub-lethal effects of zinc and mercury, including organic mercury to the freshwater alga *Chlorella*. In a recent study there appeared to be an association between Se binding proteins and a decrease in cadmium (Cd) toxicity, these binding proteins are usually rich in sulphydryl groups which may well explain the observations in *Chlorella* (Dutilleul et al. 2008). In reclamation of uranium mines there was present a growing risk of toxic levels of Se being released as a secondary problem to uranium toxicity (Sharmasarkar and Vance 2002). Finally, in phytoremediation of sites from mercury and organomercurials, Bizily et al. (1999) demonstrated that volatilisation of Hg was important and was a process similar to Se volatilisation. The genes for Hg volatilisation have been cloned and transgenic plants have been successfully used in phytoremediation; this appears to be a system in many ways similar to what is being proposed for Se phytoremediation (Rugh 2001).

## 3 Biochemistry

### *3.1 Se as an Essential Element*

There is some evidence that Se may be required for growth and development in algae, but the question of Se being an essential element (micronutrient) in higher plants remains unresolved (Yokata et al. 1988; Whanger 2002; Pilon-Smits et al. 2009). In Se accumulating plants, indications are that Se may be required for maximum growth potential, especially those endemic to seleniferous soils (Broyer et al. 1966; Broyer et al. 1972). Even in the best studied Se accumulating plant *Astragalus pectinatus* the results of additional Se application in experiments have had differing results (Shrift 1969; Stadtman 1990). It is fair to point out that other nutrients can complex the situation such as phosphates and sulphates, however the experiments so far have not used controls where residual Se is not present at all; and indeed such experiments may be near impossible to perform (Forshhammer and Boek 1991; Stadtman 1996). This is simply because there will always be trace amounts of Se in plants, coming from impurities in the nutrients used or even coming from the atmosphere.

An alternative approach to try to resolve essentiality was to try to detect Se incorporation into Se dependent enzymes, with an integral SeCys residue as present in animals and bacteria (see Sect. 3.3) (Axley et al. 1991). To conclude, the evidence so far from molecular studies available is quite strong that there is no clear evidence for essential selenoproteins in higher plants, but part of the machinery for the synthesis of selenoproteins may be present in plants (see Sect. 4.2) (Berry et al. 1991; Berry et al. 2001).

## 3.2 Se Assimilation

Higher plants metabolise Se via the sulphur assimilation pathway. Most of the sulphur assimilation pathway is well characterised and described for Se non-accumulator plants. The various biochemical steps in this pathway are described below (Zayed et al. 1999; Sors et al. 2005b).

**(a) ATP sulphurylase** – Selenate is absorbed by roots via the sulphate transporters (Fig. 10.1) and is usually transported through the xylem without modification to the leaves. Once selenate is inside leaves it enters the chloroplasts where it is metabolised by the enzymes of sulphate assimilation. The first, most critical and rate limiting step is the reduction of selenate to APSe by ATP sulphurylase (Burnell 1981), which is accumulated in the chloroplasts. However, if the same plants are supplied with selenite, organo-Se compounds similar to SeMet are assimilated. De-topped plants supplied with selenate accumulated only selenate in the roots; strongly supporting that the chloroplasts are the sites for ATP sulphurylase activity and selenate reduction (Shaw and Anderson 1972; Pilon-Smits et al. 1999).

**(b) Reduction of adenosine 5'-phosphoselenate (APSe) to selenide ($Se^{2-}$)** – The next series of metabolic steps where evidence is available is that APSe can further be reduced to selenide ($Se^{2-}$) via two pathways; one enzymatically and the other non-enzymatically (Fig. 10.2a):

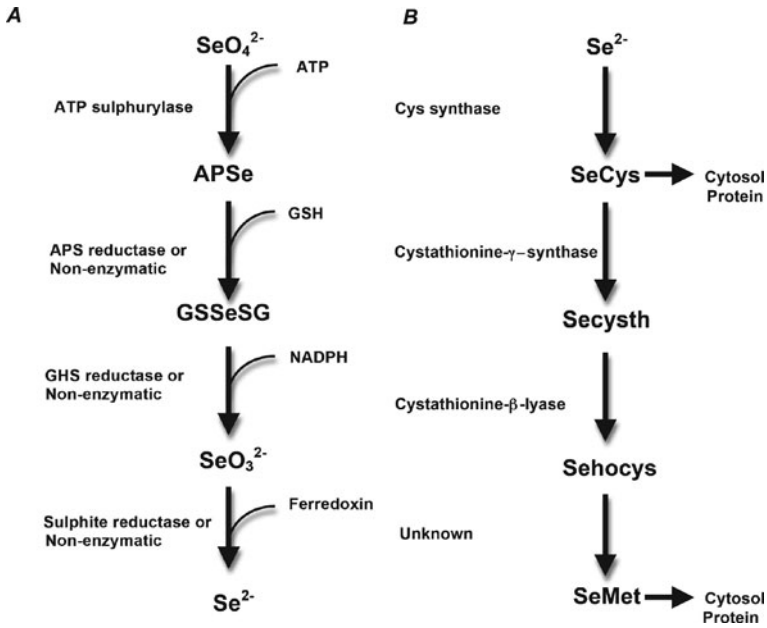


**Fig. 10.2** **a** Pathway for selenate activation and reduction to selenide, which can be either enzymatic or non-enzymatic. **b** Pathway of selenide conversion to selenocystein (SeCys) and/or selenomethionine (SeMet) and incorporation of both into proteins

1. *Non-enzymatically* – with the aid of GSH, NADPH and FADH; however GSH reductase (i.e., glutathione reductase) may be necessary as a side reaction (Anderson 1993; Ng and Anderson 1979); and
2. *Enzymatically* – via APS reductase and sulphite reductase; although one non-enzymatic step may also be required (Arvy 1997; Terry et al. 2000).

The intermediate compound selenite ($SeO_3^{2-}$) can also undergo other transformations besides its final assimilation and reduction to selenide, and enter alternate pathways. This is achieved non-enzymatically by reduction to GS-Se-SG, which is reduced to the selenol (SeGSH). SeGSH is glutathione conjugated selenide. For example plants supplied with selenite can oxidise Se to selenate (Ng and Anderson 1979); a sort of reverse reaction to normal Se assimilation.

## *3.3 Incorporation of Se into Protein*

It is proposed that plants like bacteria incorporate and assimilate SeCys specifically into protein, or after it is metabolised to SeMet. It is likely that this process also occurs in the chloroplasts. In both cases Cys synthase converts $Se^{2-}$ to SeCys, which can be a reverse reaction if the enzyme SeCys lyase is present. SeCys is converted to Secysth by the enzyme cystathionine-γ-synthase, then to Sehocys by another enzyme cystathionine-β-lyase, and finally to SeMet by what is as yet an unknown mechanism (Fig. 10.2b). Finally, either a direct or an indirect pathway of incorporation into proteins takes place for both SeCys and/or SeMet (Foyer and Halliwell 1976; Goutierrey-Marcos et al. 1996):

**(a) Direct** – SeCys is incorporated via a specific SeCys t-RNA into the selenoproteins.

**(b) Indirect** – SeCys is converted to SeMet as above (Fig. 10.2b), and a specific SeMet t-RNA incorporates SeMet into selenoproteins.

## *3.4 Localisation of Se Pathways*

A summary of the cellular and sub-cellular localisation of the enzymes and metabolites in the selenium assimilation pathway are given below:

**(a) Chloroplasts** – for the selenate reduction pathway all enzymes and metabolites have been localised in chloroplasts, wether the reactions are enzymatic or non-enzymatic. Cys synthase and maybe also cystathionine-γ-synthase and cystathionine-β-lyase are localised in the chloroplast. At least until the synthesis of Sehocys most reactions occur in chloroplasts (Kim and Leustek 1996; Setya et al. 1996; Ravanel et al. 1998; Turner et al. 1998).

**(b) Cytoplasm** – SeMet production from Sehocys and methylation of SeMet to SeMMet, DMSeP and DMSe are thought to occur within the cytoplasm (Fig. 10.3a) (James et al. 1995; Terry et al. 2000).

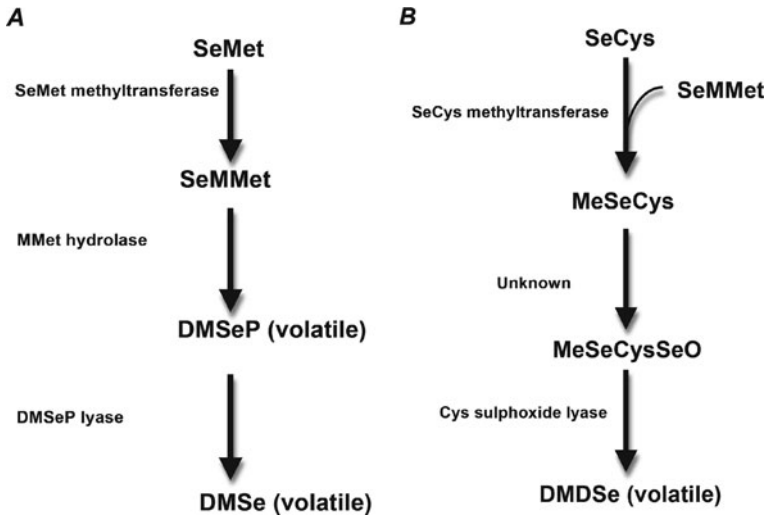


**Fig. 10.3** **a** Pathway for the production of volatile forms of Se, DMSeP and DMSe from selenomethionine (SeMet). **b** Additional pathway of production of the volatile DMDSe from selenocysteine (SeCys)

**(c) Selenium accumulator plants** – the pathway for assimilation of inorganic Se is thought to be mostly the same as described above for Se non-accumulator plants. However Se accumulators differ in that they metabolise the SeCys primarily into various seleno amino acids which are not incorporated into essential proteins. The pathway by which these Se amino acids are synthesised is probably similar to sulphur amino acids (Nigam et al. 1969; Peterson and Robinson 1972).

In the Se hyperaccumulating plants *Astragalus bisulcatus* and *Stanleya pinnata*, elemental Se was localised ultrastructurally by Freeman et al. (2006) and its distribution and chemical forms differed considerably. In *A. bisulcatus* Se was predominantly accumulated in the trichomes of young leaves, and the Se was mostly in the organic form of MeSeCys and γ-glutanyl-MeSeCys. In young leaves only 30% maximum was in the form of inorganic Se (i.e., selenate or selenite). In *S. pinnata* the Se was mostly accumulated near the leaf edges and surface globular structures in epidermal cells; most of the Se was in the form of MeSeCys (Fig. 10.3b). This was in contrast to non-accumulator plants like *A. thaliana* where most of the Se was present in the inorganic form in the vascular tissues and mesophyll cells. In hyperaccumulating plants the Se is mobile in both the xylem and phloem of young leaves, and compartmentation into organoselenium in specific organs and tissues appears to be a unique property of Se hyperaccumulator plants (Freeman et al. 2006).

# 4 Molecular Biology

## *4.1 Sulphate Transporters*

Initial research on yeast enabled the first sulphate transporter genes to be cloned in plants. These were identified as important in conferring resistance to high concentrations of selenate. Using first strand complementation between yeast and plants three genes (*SHST1, SHST2* and *SHST3*) encoding sulphate transporters were isolated in a legume (*Stylosanthes amata*) (Breton and Surdin-Kerjan 1977; Smith et al. 1995), and another gene (*HUST1*) was isolated from barley (Smith et al. 1995; 1997). Amino acid sequence and protein structural analysis suggested that the transporters contained multiple (up to 12) membrane spanning domains. Using highly conserved cDNA regions, cDNA homologous to the sulphate transporters have been isolated in *Arabidopsis*, Indian mustard, soybean and corn (Davidian et al. 2000). Consistent with the two main classes of transporters in plants and other organisms, gene families for these have also been identified:

***SHST3* gene family for low affinity transporter (LAST)** – which is expressed in both the roots and shoots, and this appears to be the main transporter gene for intracellular transport from the apoplast to the symplast. This transporter gene is modulated strongly by the sulphur status of plants and elevated GSH down-regulate transcription of the genes.

***SHST1* and *SHST2* gene families for high affinity transporter (HAST)** – which is expressed primarily in the roots, and is primarily responsible for the accumulation of sulphate from the soil to the root.

***SHST1/2*** – over-expression of these genes increased selenate accumulation by at least two fold in Indian mustard, however most of the Se was accumulated and retained in the roots.

***SHST3*** – over-expression of this gene did not significantly lead to an accumulation of selenate in plant roots, but rather allowed Se to be translocated throughout the plant.

Selenite uptake appears not to be modulated by transporters in the membranes of plants. However selection which resulted in an *A. thaliana* '*sel*' selenite mutant were found to contain less of the sulphate transporter gene *Sultin 1* in the root cortex (Shrift and Ulrich 1976; Abrams et al. 1990). This gene was found to be similar to the *SHST1* gene involved in transporting of both sulphate and selenate from the soil to the root. There are also other sulphate transporter genes (e.g., *Sultin 2* and *Sultin 3*) reported but their role in Se transportation and Se tolerance is not as well described (Table 10.3). From the few studies so far it is highly likely that in Se hyperaccumulating plants the inducible high affinity transporter (HAST) is perhaps simply more selective for selenate rather than for sulphate (Terry et al. 2000).

## 4.2 Genetic Code and Se Proteins

The incorporation of the active seleno amino acid SeCys into essential selenoproteins is a co-regulation process directed by a UGA codon. UGA normally functions as a universal terminating codon (one of three) present in higher plants (Boek et al. 1991). In order for the process to occur and for integration into proteins to proceed, both specific secondary structural elements in the mRNA and a unique SeCys-charged tRNA that contains the UGA anti codon are required (Stadtman 1996). A key reaction is the activation of selenide to form selenophosphate by the enzyme selenophosphate synthase. Selenophosphate is the Se donor for the conversion of the serine binding tRNA to the SeCys binding tRNA (Stadtman 1996).

Attempts at definitively ascertaining if selenoproteins are present in plants have yielded differing and inconclusive results. Sabeh et al. (1993) found a 6 KDa tetrameric protein in *Aloe vera* which they claim is the selenoprotein GSH peroxidase (GPX). Molecular evidence also suggests that although GPX like enzymes are present in higher plants they appear not to be selenoproteins (Anderson 1993). Peptide sequencing of purified proteins have confirmed that Cys and not SeCys is present in the active site for most of these plant GPX like enzymes. However there appears to be part of the machinery for the synthesis of selenoproteins in plants in that the UGA decoding tRNA has been demonstrated in beet and algae (Hatfield et al. 1992; Eshdat et al. 1997).

## 4.3 Key Enzyme Genes

**ATP sulphurylase** – there is experimental evidence supporting selenate is transported into the chloroplast upon uptake, where sulphate and probably selenate assimilation takes place. Mutation studies suggest that increasing expression of genes encoding ATP sulphurylase can increase selenate tolerance of plants up to ten-fold (Pilon-Smits et al. 1999). In addition, with the overexpression of ATP sulphurylase the biosynthesis of organoselenium compounds is maximised, allowing cells to tolerate increased levels of Se because levels of selenate have been reduced (Leustek et al. 1994). Overexpression of an ATP sulphurylase gene (*APS1*) in Indian mustard produced a two-fold higher accumulation of glutathione, and a 2-3 fold increase in total Se content of leaves. Almost the same effects were found in *A. thaliana* of increase Se content with overexpression of an isoform of the gene *APS2*, however in tobacco overexpression of this gene had no significant effects (Saito et al. 2000). Sors et al. (2005a) demonstrated in *A. thaliana* that overexpression of *APS1* decreased Se levels and Se tolerance. A number of subsequent studies detailed in Table 10.3 have confirmed the important role of ATP sulphurylase for increasing tolerance to Se in a number of transgenic plants.

**Selenocystein methyltransferase** – selenocystein methyltransferase (*SMT* genes) is an important enzyme in Se hyperaccumulating plants, in that large amounts of Se methyl protein are produced, and the enzyme selenocysteine methyltransferase catalyses the methylation of SeCys to MeSeCys. One of the earliest molecular

transformation reports by Lyi et al. (1995) was using this gene, where the *SMT* gene from *A. thaliana* was transferred to *B. oleracea* and affected Se levels in transformed plants. The enzyme has also been cloned in *Astragalus bisulcatus* and overexpression of this enzyme in *Astragalus* leads to both MeSeCys and MeCys synthesis, suggesting the enzyme can methylate both (Van Huysen et al. 2003). Overexpression of *SMT* in *A. thaliana* and *B. juncea* increased foliar and plant tissue Se levels, and increased tolerance to selenite, however *SMT* expression had no significant effect on selenate tolerance (summary in Table 10.3). The *SMT* protein has been characterised and is 65–70% structurally similar to the enzyme homocysteine methyltransferase (*HMT*) from *A. thaliana* and rice (*O. sativa*) (Ellis et al. 2004). Together the evidence suggests that *SMT* and *HMT* have similar structure and function; as well as their Se homologues. This may be an effective sink for both Se and S in plants, however it cannot explain the preference of *Astragalus* hyperaccumulating plants for Se over S.

**APS reductase** – the constituative expression of APS reductase (*PaAPR*) was investigated and isolated from the bacterium *P. auruginosa* and expressed in *A. thaliana*. There was increased sulphate reductive capacity and accumulation of reduced inorganic and organic forms of sulphur (Bruhl et al. 1996). When treated with selenate, plants increase selenate reduction (65–80%) suggesting it had the capacity to reduce APSe. This was accompanied by a decrease in foliar Se and increased selenate tolerance (Table 10.3). In *Astragalus*, APS reductase activity was similar in non-accumilating and hyperaccumulation species.

**Serine acetyltransferase** – serine acetyltransferase (*SATm*) is a key enzyme leading to Cys biosynthesis, and this enzyme which in many reports is localised in the mitochondria plays an important regulatory role. In transgenic tobacco where *SAT* overproduction was present, results indicated a drastic increase in o-acetyl serine (OAS) and Cys, and glutathione levels six times higher were recorded (Losi and Frankenberger 1997). However the plants showed no difference in Se accumulation or tolerance (Table 10.3). As well, the hyperaccumulator *Astragalus* was not correlated to higher expression of *SAT*, and it appears that Cys synthesis does not limit selenate accumulation.

**Selenocysteine lyase** – this enzyme in Se assimilation has been cloned and expression of this gene in *B. juncea* originally sourced from *A. thaliana* appeared to reduce selenate toxicity, and Banuelos et al. (2007) attributed this to a reduction in incorporation of Se into proteins (see Table 10.3). The gene used in this study may well be similar to the *AtCpNifS* chloroplast gene used below by Van Hoewyk et al. (2005).

**Selenocysteine transferase** – this enzyme was also cloned and expression of this gene in *B. juncea* sourced from *A. thaliana* appeared to have little effect on selenate toxicity but had a small effect on selenite toxicity (Banuelos et al. 2007). The gene describe here may well be similar to the *SMT* gene family used above (Table 10.3), but its full name was not used in the report.

**Cystathionine-γ-synthase** – another important enzyme in Se assimilation has been cloned and overexpression of *CyS* genes in *B. juncea* lowered Se levels in shoots and increased Se tolerance (Table 10.3).

**Chloroplast selenocysteine lyase** (*AtCpNifS*) – genes for a chloroplast protein-like SeCys lyase enzyme have been cloned. When this gene was overexpressed in *A. thaliana* it enhanced selenate tolerance by reducing Se uptake into proteins (Van Hoewyk et al. 2005).

**Selenium binding proteins** (*SBP123*) – a more distant related family of genes that induce higher levels of binding polypeptides and proteins, well studied in *A. thaliana*. It was recently found by Dutilleul et al. (2008) that expression of what was considered specific binding proteins for Se also conveyed tolerance to the heavy metal cadmium (Cd); most likely also by binding this heavy metal (Table 10.3).

**Sulphate proton transporter genes** – The *Sultr 123* family of genes regulates sulphate transporters, and by association may also regulate Se transportation. Lydiate et al. (2007) using 'knock-down' technology in *A. thaliana* of *Sultr 123* genes reduced HAST transportation of Se, but had little effect on selenite transportation (Table 10.3). The *Sultr* family of genes are likely to be similar to the *SHST* family of genes described before.

For such advancement in molecular and genetic studies as outlined above, it must be pointed out the very important contribution of research by Zeibur and Schrift (1971) where they successfully initiated in tissue and callus culture various species of *Astragalus*. Without the aid of tissue culture, mutagenic and genetic studies on critical enzymes of Se assimilation in different species of *Astragalus* would have been difficult. Another important molecular study was that of Wang et al. (1999) where they clearly demonstrated Se tolerance could be increased via simple selection methods. Analytical methods such as the use of radioactive Se, enzymatic detection assays, immunoblotting and two-dimentional (2-D) electrophoresis separation were also used in this study, which have become standard techniques in later research.

In the future, molecular investigations on Se will need to follow the lead of three other important investigations, which have laid the foundation for more detailed research:

**(a) Mapping of quantitative trait loci (QTL)** associated with Se tolerance, like the study of Zhang et al. (2006) and Zhang et al. (2007) where selenate tolerance was linked to root growth and epistatic to other important traits, and these genes could be mapped on different chromosomes of *A. thaliana*.

**(b) Microarray analysis** to compare many up-regulated and down-regulated genes and metabolites between different Se performing clones, like in the study of Tamaoki et al. (2008), where it was found that reactive oxygen radicals and plant hormones were important in Se tolerance.

**(c) Proteomic analysis** to confirm and detail molecular differences in polypeptide and protein fragments where up-regulated and down-regulated genes and metabolites are involved, and even if the proteins identified contain seleno amino acids or not; like the clinical studies reviewed by El-Bayoumy and Sinha (2005).

### *4.4 Methylation and Volatilisation*

After SeMet is synthesised it can be methylated and converted to dimethylselenide (DMSe) which is the major volatile Se compound in non-Se accumulating plants. The enzymatic steps are well known (Giovanelli et al. 1980; Bourgis et al. 1999) (Fig. 10.3a), however no detail knowledge of the enzymes, except SeMet hydrolase at the molecular level have been investigated. Plants can also volatilise Se as dimethyldiselenide (DMDSe) via oxidative and subsequent methylation with an intermediate DMSeP which is also volatile. The enzymatic and biochemical steps are also well known but no molecular biology knowledge is available (Fig. 10.3b).

## 5 Phytovolatilisation

### *5.1 Se Volatilisation*

In summary, SeMet may be methylated to Se-methyl-Met (SeMMet) by a series of enzymatic steps which eventually can produce DMSe, or indirectly via the intermediate phosphorylated DMSeP. In either case DMSe (Fig. 10.3a) is produced, and it can be volatilised with the aid of the enzyme DMSeP lyase thought to exist in plants (Hanson et al. 1994; Hanson et al. 1997). By analogy with the production of DMS (dimethyl sulphide) in plants DMSP occurs in chloroplasts. However since roots volatilise more DMSe than shoots or leaves it must be assume that all the enzymes necessary, and especially SMMet hydrolase and DMSeP lyase are also present in roots. The synthesis of SeMet appears to be rate limiting for Se volatilisation (Hanson et al. 1997) and the conversion of SeMet to DMSeP is also rate limiting in plants (Hanson et al. 1997). In accumulator plants in particular methylation to DMSe is abundant before it is volatilised. Similarly, all of the enzymes and steps for production and volatilisation of DMDSe from SeCys are known, except the enzyme that converts MeSeCys to MeSe CysSeO, or this step may be a non-enzymatic step (Fig. 10.3b).

### *5.2 Variation Amongst Plants*

The rate of Se volatilisation varies widely amongst plant species. Rates can be from a high of 200–300 mg Se $m^{-2}$ leaf area $day^{-1}$ in rice, broccoli, cabbage and *Astragalus* to less than 15 mg Se $m^{-2}$ leaf area $day^{-1}$ in sugar beet, bean, lettuce, tomato, alfalfa and tall fescue. In trials, wetland plants showed a 50-fold variation in Se volatilisation, with a low rate of 1 mg Se $kg^{-1}$ dry weight $d^{-1}$ attained for selenate, to a higher rate of 4 mg Se $kg^{-1}$ dry weight $d^{-1}$ for selenite in *Azzola*. The plant *Salicornia bigelovii* had a high rate of Se volatilisation of 420 μg Se $m^{-2}$ soil $d^{-1}$, and was between 10 and 100 times greater than other species tested; including

salt grass, cord grass, cotton, *Eucalyptus* and canola (Duckart et al. 1992; Terry and Lin 1999).

## 5.3 Plant/Microbe Interactions

Bacteria, fungi and algae can assimilate and volatilise Se independently of plants; and the rates achieved can be considerably higher than in plants. The question therefore arises in Se volatilisation is how independent are plants in volatilising Se by the presence of microbes in the rhizosphere. An early indication of some dependence by plants on microbes was obtained when de-topped roots were treated with antibiotics (Terry et al. 1992; Brady et al. 1996; De Souza and Terry 1997; Pilon-Smits et al. 1999). The rate of Se volatilisation was reduced by antibiotics by as much as 95% for selenate supplied broccoli. Subsequent research was done to try to resolve this question with sterile and non-sterile tissue culture plants. Using Indian mustard it was shown that Se volatilisation did require a rhizosphere to volatilise substantial Se from selenate and selenite; but this was not the case when SeMet was added (Rael and Frankenberger 1996; Fan et al. 1997).

The role of the rhizosphere microbes appeared to be somewhat specific for selenate and its uptake, by producing heat labile compound(s) that were proteinaceous in nature; possibly the amino acid derivative o-acetylserine (OAS) and the amino acid serine which can stimulate the uptake of selenate by the sulphate transporters. There was no such stimulation with selenite supplied plants, and indications were that the rhizosphere organisms aided in the production of organic Se compounds like SeMet, which can be converted to DMSeP and DMSe, and both of these compounds are more readily volatilised (Thompson-Eagle et al. 1989; Zayed et al. 1998).

## 5.4 Environmental Factors

The ability of plants to volatilise Se is influenced by the concentration of Se around the roots and the chemical form of Se supplied. There was a direct linear relationship between an external Se concentration and internal plant tissue concentration of Se in Indian mustard supplied with selenate or selenite (De Souza et al. 1999). Se volatilisation was also correlated to plant tissue concentrations, and selenite treated plants released 10–15 times more Se than plants supplied with selenate. However plants supplied with SeMet volatilised Se at an even higher rate; but plants supplied with DMSeP volatilised Se at the highest rate recorded (Terry et al. 1992). These findings were consistent with studies described before for aquatic plants in constructed wetlands (Terry 1998).

An important environmental factor in volatilisation of Se is the concentration of sulphate compared to selenate in the soil. Se volatilisation can be inhibited strongly by the presence of sulphate in the range of 0.25–10 m*M*. Rates of volatilisation decreased from 97 to 14 $\mu$g Se $m^{-2}$ leaf area $day^{-1}$ with the higher sulphate supply

(Zayed et al. 1998). The rate of inhibition generally decreases with an increase in the S:Se ratio in plant tissue. The inhibition of volatilisation suggests that sulphur compounds out compete Se compounds for the active sites of the enzymes responsible for Se volatilisation. In the field, rates of volatilisation vary enormously, and also vary with the time of the year (Martens and Suarez 1997). Se volatilisation is at its highest rate in spring and early summer. In wetlands, Se volatilisation is dependent on many parameters, like Se concentration, water sediment, the plant used, microbial biomass in sediment, pH, salinity, dissolved oxygen, depth and temperature. However the most important factors appear to be water temperature, Se concentration in roots and microbial biomass in the sediment (Hanson et al. 1997; Terry and Lin 1999).

## 6 Phytoremediation

### *6.1 Process*

Low level large scale contamination presents monumental economic and logistical barriers to effective, timely treatment. A number of technologies have been successfully applied, and all fall into the two broad categories below:

**Engineering based technologies** – which can be aggressive and are usually applied to cleanup more acute polluted point sources. These can be not cost effective or even environmentally justified for marginally affected sites. The methods can be diverse but usually include excavation and entombment or variations of these methods Lynch and Moffat (2005). The methods are not likely to diminish or alleviate the hazardous material, and more importantly they cannot reduce landfill capacity. Engineering based approaches are usually applied to where more rapid responses are required but can cause secondary problems in the long term (Pilon-Smits 2005; Banuelos 2006). These engineering methods and their possible application to Se remediation will not be covered in this review.

***In situ* biological remediation** – could be a cost effective and more appropriate corrective option for treatment of wide-spread, low impact contamination (Banuelos 2001). The methods fall into two sub-categories of:

*Bioremediation* – a microbial induced process, and
*Phytoremediation* – which refers to a plant based clean-up processes.

### *6.2 Plant Species*

A variety of plant taxa possess a remarkable natural ability to accumulate metals (phytoextraction) or even degrade organic compounds (phytodegradation). Superior Se phytoaccumulating species of plants have been characterised, identified and

studied at the physiological, biochemical and molecular level. Even more, a selected few of these important plants have been well described at the molecular and genetic level, and a very small number have been genetically manipulated. For example, Banuelos et al. (2002) have identified and transformed the functional trait (actually a key enzyme) from a Se hyperaccumulating species (*A. bisulcatus*) to the non-accumulator *A. thaliana*; conferring increased Se tolerance and some increase accumulation of Se (see Table 10.3). Metal hyperaccumulating plants and their identification have been recognised for a relatively long time (Berken et al. 2002), and have been used in different ways by researchers and ecologists. Some of the ways metal and metalloid hyperaccumulating plants have been used include:

**Phytomining** – historically metal hyperaccumulating plants were only recognised for their ability to identify sites or areas useful as possible mining sites, mostly of sought after deposits of metals (phytoprospecting) and recovery of the metals (Baker et al. 2000).

**Revegetation** – more recently plants that can survive high metal content have been used increasingly in revegetation projects, some necessary by legislation, and yet others done for aesthetic purposes, as for example barren, eroding mining or industrial impacted soils. Recovery of metals was not a primary objective (i.e., as in phytomining) as it was deemed that recovery was too expensive and uneconomic (Sors et al. 2005a). However these practices and other technologies have lead to the 'invention' of more refined phytoremediation techniques.

**Metal recovery** – plant based recovery of soil based metals and their reuse has been described only for nickel (Ni) and thallium (Th); which have high economic value. Other toxic metals for example like mercury (Hg), lead (Pb), arsenic (As), cadmium (Cd) and caesium (Cs) have little economic value and are also extremely toxic; these must be processed as hazardous waste and so far have not been proposed to be used in conjunction with accumulation in plants (Freeman et al. 2004).

**Biological beneficial minerals** – essential minerals could be good candidates for combined phytoextraction and use in for example dietary supplements. These include zinc (Zn), iron (Fe) and selenium (Se), which have been used in crop fortification for increased essential mineral enrichment of edible crops (Finley 2005). Indian mustard (*B. juncea*), *Astragalus* species and a number of other crops and vegetable species have been fortified for Se for many years now (a list is presented in Table 10.2) (Mayland et al. 1989; Parker et al. 1991).

## *6.3 Para-Phytoremediation*

Such mixed-benefit strategies as described just before should be considered to be 'para-phytoremediation', which combines and identifies the useful part of the remediation method in plants with their ability to detoxify the environment in which plants are grown (Wu et al. 1988; Wu 2004). There may be other products that could be obtained from plants loaded with potentially toxic and valueless metals and metalloids, apart from nutritional enhancement for essential micronutrients

and environmental detoxification. One such benefit proposed is energy production which accompanies incineration, and is a procedure required to process and dispose of hyperaccumulating plant biomass. Another possible product in the case of Se could be in the extraction of biopharmaceutical compounds used in cancer treatment (Banuelos 2006). The attraction and benefits of these proposals are that these so called 'crops' could be grown on otherwise non-productive lands for profit; and these could be strong incentives to cost-effective treatment of toxic area for not only energy, but paper, fibre, building materials and health supplement/treatment.

### *6.4 Problems*

An obvious concern over phytoremediation techniques, especially in using genetically modified plants is the possible transfer of undesirable traits to elite plants and crop cultivars for agriculture (Hanson et al. 1997; Terry et al. 2000). The concern over hyperaccumulation and high levels of for example Se during uptake into plants may limit the use of phyto-crops for food or animal consumption. However technology exists to identify the fate of most of these toxic compounds, and their toxicity; as demonstrated with the development of chemo preventitive enriched Se accumulating (fortified) edible crop plants like potato, radish and other vegetables in Australia, UK, USA and other parts of the world (Table 10.2) (Broadley et al. 2004; Lefsrud et al. 2006; Pedrero et al. 2006; Haug et al. 2007; Zhao et al. 2007).

A major environmental problem is how to clean-up Se from constructed wetlands and their waters. An affective solution appears to be to use 'artificially constructed wetlands'. Up to 90% of Se from oil refinery effluent has been shown to be removed by wetlands, and Se was substantially contained in the sediment. But a considerable amount was present in plant tissue, and a reasonable amount also volatilised into the atmosphere (10–30%). Wetland efficiency for removal of Se depends on the most suitable plant species planted and some species like cattail grass (by size of its biomass) and widgeon grass (by amounts hyperaccummulated) removed the most Se in trials so far (Banuelos 2006; Nyberg 1991). A full review of Se removal by constructed wetlands is presented by Wu (2004), and it will not be dealt with further in this review.

## 7 Conclusions/Future Directions

World selenium (Se) resources need to be managed so that this non-renewable vulnerable resource is not squandered. Se uptake, mobilisation and assimilation are quite well understood and are similar to sulphur, however there are some steps not well understood, especially enzymatic and non-enzymatic steps leading to the reduction to selenide. Se hyperaccumulating plants do have differences in uptake and sequestration of Se which require more investigations, and essentiality of Se to

higher plants also needs to be resolved. Growth potential of Se plants as agricultural crops for biomass production and identification of the chemical species of Se present and their quantification in plants is necessary for any use in health supplementation. Seleniferous soils are potentially useful in their use, but the soils need to be better identified and field testing needs to be done before they may be considered potentially usable for an intense agricultural system of farming. It is also clear that just simple biofortification of crops needs to be considered carefully for value and effects. Perhaps a new method combining the use of Se-enriched sprouts (i.e., young tender shoots) provided through the germination of seeds of selected plants in Se rich soils is an interesting new concept worth considering and trialling.

Molecular studies and overexpression of genes encoding proteins involved in Se uptake, transport and assimilation have been reported, and we can still expand on these types of experiments and observations. In this way further strategies for genetic engineering of Se accumulation, transformation and toxicity will become evident, and the use of transgenic plants for use in a variety of ways could be evaluated. Phytoremediation offers a cost effective and environmentally friendly alternative or complementary technology to conventional bioremediation techniques. However the underlining biological processes of phytoremediation are still largely unknown in many cases, and important areas which need more detail investigations are plant-microbe interactions, mechanisms of degradation and transformation, volatilisation, chelation, binding and detoxification. The feasibility of mixed-use strategies for phytoremediation is worth considering with the use of genetically improved phytocrops in Se enriched soils. In this regard there is value in enhancement of traits in plants useful in phytoremediation such as high biomass and growth potential in seleniferous soils, which might otherwise be considered agriculturally non-productive land. Se-hyperaccumulating plants (wether naturally occurring or transgenic plants) have possibilities in that they combine pollutant decontamination with production of a product with beneficial properties to humans and animals.

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