Extraordinarily high leaf selenium to sulphur ratios define "Se-accumulator" plants

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Abstract

- *Background:* Selenium (Se) and sulphur (S) exhibit similar chemical properties. In flowering plants (angiosperms) selenate and sulphate are acquired and assimilated by common transport and metabolic pathways. It is hypothesized that most angiosperm species show little or no discrimination in the accumulation of Se and S in leaves when their roots are supplied a mixture of selenate and sulphate, but some, termed Se-accumulator plants, selectively accumulate Se in preference to S under these conditions.
- *Methods:* This paper surveys Se and S accumulation in leaves of 39 angiosperm species, chosen to represent the range of plant Se accumulation phenotypes, grown hydroponically under identical conditions.
- *Results:* The data show that, when supplied a mixture of selenate and sulphate, (1) plant species differ in both their leaf Se ($[Se]_{leaf}$) and leaf S ($[S]_{leaf}$) concentrations, (2) most angiosperms show little discrimination for the accumulation of Se and S in their leaves and, in non-accumulator plants, $[Se]_{leaf}$ and $[S]_{leaf}$ are highly correlated, (3) $[Se]_{leaf}$ in Se-accumulator plants is significantly greater than other angiosperms, but $[S]_{leaf}$, although high, is within the range expected for angiosperms in general, and (4) the Se/S quotient in leaves of Se-accumulator plants is significantly higher than other angiosperms.
- *Conclusion:* The traits of extraordinarily high $[Se]_{leaf}$ and leaf Se/S quotients define the distinct elemental composition of Se-accumulator plants.

Key words: Angiosperm, *Astragalus, Brassica,* leaf, mineralogy, selenium, sulphur.

INTRODUCTION

Sulphur (S) and selenium (Se) are both naturally occurring Group VIA elements and exhibit similar chemical properties (Dhillon and Dhillon, 2003; Sors *et al*., 2005*b*; Broadley *et al*., 2006; White *et al*., 2007). Although S is an essential element for plant nutrition (Mengel and Kirkby, 2001), Se does not appear to be required for plant growth or reproduction, and excessive Se concentrations can be toxic (Dhillon and Dhillon, 2003; White *et al*., 2004, 2007; Broadley *et al*., 2006).

Species of flowering plants (angiosperms) differ in their abilities to accumulate S (Hurd-Karrer, 1937; Willey and Wilkins, 2006) and Se (Rosenfeld and Beath, 1964; Brown and Shrift, 1982; Dhillon and Dhillon, 2003; White *et al*., 2004). For example, brassicas and alliums accumulate unique organo-S compounds in their tissues and, consequently, have higher tissue S concentrations than many other plants grown under the same conditions (Mengel and Kirkby, 2001; Willey and Wilkins, 2006). Similarly, although there is little variation in shoot Se concentrations ($[Se]_{shoot}$) between angiosperm orders, there is considerable variation in $[Se]_{shoot}$ between plant species within orders (White *et al*., 2004). Indeed, angiosperms have been divided into three ecological types of 'non-accumulator', 'Se-indicator' and 'Se-accumulator' plants (Rosenfeld and Beath, 1964; Shrift, 1969; Brown and Shrift, 1982; Dhillon and Dhillon, 2003; White *et al*., 2004). Non-accumulator plants are unable to grow on seleniferous soils and Se is toxic at tissue concentrations as low as 10 - 100 μg Se g^{-1} dry matter (Rosenfeld and Beath, 1964; White *et al*., 2004), whereas Se-indicator plants can colonise both non-seleniferous and seleniferous soils and tolerate tissue Se concentrations approaching 1000 μg Se g^{-1} dry

matter (Rosenfeld and Beath, 1964; Moreno Rodriguez *et al*., 2005). The distribution of Se-accumulator plants is generally restricted to seleniferous soils, where their shoots can contain up to 20 - 40 mg Se g^{-1} dry matter (Rosenfeld and Beath, 1964; Brown and Shrift, 1982). However, this ecological classification confounds the traits of Se accumulation and Se tolerance. A recent meta-analysis of literature data (White *et al*., 2004) suggests that $[Se]_{shoot}$ exhibits a continuous distribution among angiosperm species and, although Se accumulator plants generally have a higher $[Se]_{shoot}$ than other angiosperm species, it is unclear whether they form a discrete subset of angiosperm $[Se]_{shoot}$ consistent with the epithet "Se-accumulator plants".

Plants acquire Se primarily as selenate (SeO_4^{2-}), which enters root cells through high affinity sulphate transporters (HASTs) in their plasma membrane (Terry *et al*., 2000; White *et al*., 2004, 2007; Sors *et al*., 2005*b*). The genome of the model plant, *Arabidopsis thaliana*, contains 14 genes encoding sulfate transporters, and a similar number are present in the genomes of other plant species (Hawkesford, 2005; Hawkesford and De Kok, 2006). Several sulphate transporters appear to contribute to selenate uptake and accumulation. Circumstantial evidence suggests that these sulphate transporters differ in their selectivity between selenate and sulphate. Not only do different plant species (Rosenfeld and Beath, 1964; Bell *et al*., 1992; Galeas *et al*., 2007) and ecotypes of plant species (Feist and Parker, 2001) have contrasting shoot Se/S quotients when grown under the same conditions, but also the shoot Se/S quotients of a single plant genotype, such as *Arabidopsis thaliana* accession Col-5 *gl1*, changes with its complement of root HASTs (White *et al*., 2004). It is thought that Se-accumulator plants, such as *Astragalus bisulcatus* and *Stanleya pinnata*, always have shoot Se/S quotients greater than those in

the rhizosphere solution (Bell *et al*., 1992; Feist and Parker, 2001; Ellis and Salt, 2003; Galeas *et al.,* 2007), whereas all other angiosperms have similar or lower shoot Se/S quotients than those of the rhizosphere solution (Hurd-Karrer, 1937; Bell *et al*., 1992; Barak and Goldman, 1997; Kopsell and Randle, 1997, 1999; Feist and Parker, 2001; Grieve *et al*., 2001; Suarez *et al*., 2003; White *et al*., 2004; Galeas *et al.*, 2007). It is, therefore, hypothesized that the dominant HASTs of Se-accumulator plants are selective for selenate, whereas those in other angiosperm species are selective for sulphate (White *et al*., 2004; Sors *et al*., 2005*b*; Broadley *et al*., 2006). These distinct phenotypes have been reported for only a limited number of plant species, but, if the observation and hypothesis are correct, then the protein structure of the HASTs from Se-accumulator plants could be used to identify the elusive molecular determinant for the anionic selectivity of sulphate transporters.

In the present study, 39 plant species chosen to represent the range and distribution of $[Se]_{shoot}$ estimated for 185 angiosperm species in a previous literature survey (White *et al*., 2004) were grown hydroponically in a glasshouse in a solution with a complete mineral complement containing 910 μM sulphate and 0.63 μM selenate. Preliminary experiments suggested that this selenate concentration was unlikely to result in toxic tissue Se concentrations. Leaf Se concentrations ($[Se]_{leaf}$) were determined to test whether the $[Se]_{leaf}$ of Se-accumulator plants was distinct from the $[Se]_{leaf}$ of other angiosperms, and leaf S concentrations ($[S]_{leaf}$) were determined to assess whether $[S]_{leaf}$ or leaf Se/S quotients differed between Se-accumulator plants and other angiosperms. From these new analyses, it was concluded that, when grown hydroponically at low, non-toxic Se concentrations: (1) $[Se]_{leaf}$ was significantly greater in Se-accumulator plants

than in other angiosperms, (2) $[S]_{leaf}$, although high in Se-accumulator plants, is within the range expected for angiosperms in general, and (3) the leaf Se/S quotient had a discontinuous distribution among angiosperm species, with the Se/S quotients in leaves of Se-accumulator plants being significantly greater than those in other angiosperms. The latter, discrete compositional trait could be used to identify Se-accumulator species for subsequent molecular studies.

MATERIALS AND METHODS

Thirty nine plant species were grown in this study (Table 1). These were chosen to represent the range and distribution of relative shoot Se concentrations ($[Se]_{shoot}$) estimated for 185 angiosperm species in a previous literature survey (White *et al*., 2004). Seeds of *Astragalus glycyphyllos* and *Astragalus racemosus* were obtained from B and T World Seeds (Paguignan, France). Seeds of *Atriplex hortensis*, *Beta vulgaris*, *Bouteloua gracilis*, *Cucumis sativa*, *Helianthus annuus*, *Linum usitatissimum*, *Oryza sativa*, *Oryzopsis hymenoides*, *Raphanus sativa*, *Solanum melongena*, *Sporobolus airoides*, *Trifolium pratense* and *Trifolium repens* were obtained from Chiltern Seeds (Ulveston, UK). Seeds of *Agrostis stolonifera*, *Brassica arvense*, *Brassica juncea*, *Brassica nigra*, *Bromopsis inermis*, *Cynodon dactylon*, *Dactylis glomerata*, *Holcus lanata*, *Lolium multiflorum*, *Medicago lupulina*, *Medicago sativa*, *Panicum miliaceum*, *Sinapis alba*, *Sorghum bicolor* and *Trifolium subterraneum* were obtained from Herbiseed (Twyford, UK). *Solanum tuberosum* was obtained from Higgins Agriculture (Doncaster, UK). Seeds

of *Brassica carinata*, *Brassica oleracea*, *Hordeum vulgare* and *Lycopersicon pennellii* were obtained from Horticulture Research International (Wellesbourne, UK). Seeds of *Machaeranthera bigelovii* were obtained from Rocky Mountain Rare Plants (Franktown, USA). Seeds of *Astragalus sinicus* were obtained from Sheffield's Seed Company (Locke, USA). Seeds of *Machaeranthera tanacetifolia* and *Stanleya pinnata* were obtained from Western Native Seed (Coaldale, USA). Seeds were germinated, seedlings were weaned, and plants were grown in a hydroponic system using a nutrient film technique essentially as described by Broadley *et al*. (2003, 2004). The experiment was performed between July and August 2004. All seeds were germinated in the dark on filter paper moistened with deionized water at either 25 °C or 4 °C. Once a radicle was observed, individual seedlings were transplanted to rockwool plugs (2.5 x 2.5 x 4 cm; Grodan, Hedehusene, Denmark), which were placed in plastic trays containing tap water in a weaning room at 25 °C. Three to five days after transplanting, rockwool plugs containing individual plants were transferred to a hydroponic system in a glasshouse at Wellesbourne, UK. The hydroponic system comprised six covered gullies (5.15 m length x 0.11 m width x 0.05 m depth) constructed from flat-bottomed PVC guttering. The gullies were spaced 0.26 m apart (centre-to-centre). Circular holes were cut with equal spacing in the covers of the gulleys and two rockwool plugs, containing plants of the same species, were placed in each hole such that their bases rested directly on the bottom of the gully. The statistical design allocated all 39 species to each gulley in a random order. A nutrient solution made up in deionized water and containing 2 mM $Ca(NO_3)_2$, 2 mM NH_4NO_3, 0.75 mM $MgSO_4$, 0.5 mM KOH, 0.25 mM KH_2PO_4, 0.1 mM FeNaEDTA, 30 μM H_3BO_3, 0.03 mM $CaCl_2$, 10 μM $MnSO_4$, 3 μM $CuSO_4$, 1 μM $ZnSO_4$, 0.5 μM

Na_2MoO_4 and 0.63 μM Na_2SeO_4 (0.05 mg Se L^{-1}), adjusted daily to pH 6-6.4 using H_2SO_4, was circulated through the gulleys from a 200 litre tank. The total sulphate concentration in the nutrient solution approximated 910 μM (29.18 mg S L^{-1}). Solutions were replaced weekly. Analysis of fresh and spent nutrient solutions indicated that there were no significant reductions in Se or S concentrations during the experiment. The glasshouse was set to maintain temperatures of 25 °C by day and 15 °C at night using automatic vents and supplementary heating. Plant shoots from the 39 plant species were harvested during vegetative growth. Plant material from the same hole was bulked. Shoots were separated into leaves and stems, where possible, and the fresh weight (FW) of each was recorded. Samples were dried in paper bags for 72 h in a fan-assisted oven set to 80 °C. The dry weight (DW) of leaves and stems was measured and dry leaf tissue was subsequently milled to a powder. Powdered samples (0.3 – 1.0 g DW) were digested with nitric acid in closed vessels using a microwave digester. Total Se and S contents of dry leaf tissue were determined using inductively-coupled plasma emission spectrophotometry (JY24, Jobin-Yvon ISA, France). Plant material was analysed in batches and, in each batch, a sample spiked with 1 mg Se kg^{-1} DW of a calibration standard Se solution (Fisher Scientific, Loughborough, UK) served as an internal control for the analytical protocol. The recovery of exogenous Se was 103 ± 1% (mean ± SE, n = 30 samples). A preliminary examination of the data suggested that the mean values of leaf Se/S quotients were approximately normally distributed, whereas those of $[Se]_{leaf}$ and $[S]_{leaf}$ were approximately log-normally distributed. The appropriate distributions were fitted using GenStat Version 8 (VSN International Ltd, Oxford, UK) with parameter estimates of mean and standard deviation (SD). The log-normal transformations were

done to the base *e*. The goodness of fit was tested using a quantile-quantile plot with simultaneous 95 per cent confidence bands using the statistic given by Michael (1983). The expectation is that if the experimental data truly fit the distribution then all the data in 95 per cent of such plots will fall entirely within the confidence bands. In all cases there were significant deviations from the ideal when all plant species were included in the analysis. The species with the highest values were sequentially removed from all three distributions until there were no significant improvements in the goodness of fit.

RESULTS AND DISCUSSION

Thirty nine plant species were chosen to represent the range and distribution of relative $[Se]_{shoot}$ estimated for 185 angiosperm species in a previous literature survey (White *et al*., 2004). When these species were grown hydroponically in the same glasshouse environment, there were marked differences between plant species in their mean $[Se]_{leaf}$ (Table 1, Fig. 1). The mean $[Se]_{leaf}$ of 37 species (mg Se kg^{-1} DW) appeared to show a log-normal distribution (Fig. 2A, mean = 2.35 ± 0.08, SD = 0.496 ± 0.058, n = 37). There were small but significant deviations ($P<0.05$) from this distribution, but further removal of species with the highest mean $[Se]_{leaf}$ from the analysis did not continue to improve the fit. The low probabilities of the two plant species with the highest mean $[Se]_{leaf}$ being part of this distribution, *Astragalus racemosus* ($P<0.0001$) and *Stanleya pinnata* ($P<0.0001$), suggests the occurrence of at least two distinct phenotypes for $[Se]_{leaf}$. Even if these two species were to be included in the fitting procedure, the probabilities of observing such

extreme values remain low (*Astragalus racemosus*, P<0.0001; *Stanleya pinnata* P=0.011). This is consistent with previous studies leading to the hypothesis that Se non-accumulator and Se accumulator plants come from distinct populations, rather than there being a single, continuous distribution of $[Se]_{leaf}$ among angiosperm species.

The rank order for mean $[Se]_{leaf}$ of the 39 species grown hydroponically in the present study was not identical to their rank order of relative $[Se]_{shoot}$ estimated from a previous literature survey (Fig. 1). For both the hydroponic study and the literature survey it was assumed that selenate was the only form of Se taken up by the plant. The underlying reasons for the lack of concordance between this hydroponic experiment and the literature survey are likely to reflect these facts: (1) In the present study all plant species were assayed under identical conditions, whereas the meta-analysis performed in the literature survey integrated data from many studies performed under contrasting environmental conditions (White *et al*., 2004). The rank order of species for $[Se]_{shoot}$ in the literature survey could be confounded by several factors. (a) Plant species might show different Michaelis-Menten type relationships between selenate concentration in the rhizosphere and $[Se]_{shoot}$ and, if they did, the rank order of species for $[Se]_{shoot}$ might differ between experiments performed at contrasting rhizosphere selenate concentrations. (b) The uptake of selenate by different plant species might show contrasting sensitivities to environmental factors such as temperature, pH and redox conditions of the rhizosphere or the presence of competing anions like sulphate and phosphate, and, if this were true, the rank order of species for $[Se]_{shoot}$ could be influenced by growth substrate and/or composition of the rhizosphere solution. (c) In some experimental systems, Se might be present in chemical forms other than selenate, such as selenite and/or organoselenium

compounds that are taken up by roots by transport proteins other than HASTs, and the rank order of species for $[Se]_{shoot}$ might then reflect the complement of these transport proteins in different plant species. (2) When Se is supplied as selenate, there is considerable variation in $[Se]_{shoot}$ between ecotypes of wild plants (Davis 1972; Feist and Parker, 2001; Zhang *et al*., 2006*a*, *b*) and varieties of crop plants (Bañuelos *et al*., 1997, 2003; Kopsell and Randle, 1997; Pezzarossa *et al*., 1999; Lyons *et al*., 2005). Nevertheless, in both the hydroponic study reported here and in the literature survey, *Stanleya pinnata* and *Astragalus racemosus* were consistently observed to have extremely high mean $[Se]_{leaf}$. Intriguingly, the lack of concordance of the rank order of angiosperm species for $[Se]_{shoot}$ in the hydroponic study and the literature survey reported here contrasts sharply with a similar comparison of the rank order of angiosperm species for $[Ca]_{shoot}$ between a hydroponic study and a literature survey (Broadley *et al*., 2003), which might reflect (a) the quasi-linear increase of $[Ca]_{shoot}$ with increasing rhizosphere Ca^{2+} (White, 2001) and (b) the likelihood that Ca is taken up by plants solely as Ca^{2+} (White and Broadley, 2003).

Significant differences in mean $[S]_{leaf}$ were observed among plant species grown hydroponically in the same glasshouse environment (Table 1). The mean $[S]_{leaf}$ (g S kg^{-1} DW) of the 39 species studied here appeared to belong to a log-normal distribution (Fig. 2B, mean = 1.87 ± 0.08; SD = 0.52 ± 0.06, n = 39). This is consistent with the distribution of relative $[S]_{shoot}$ in 121 angiosperm species obtained in a recent literature survey (Willey and Wilkins, 2006). Although not as extreme as for $[Se]_{leaf}$, there were significant deviations from the log-normal distribution ($P<0.01$). The two species with the highest mean $[Se]_{leaf}$, *Astragalus racemosus* and *Stanleya pinnata*, also had the third

and second highest mean $[S]_{leaf}$, respectively. *Brassica oleracea* had the highest mean $[S]_{leaf}$. Exclusion of these three species from the fitting resulted in a log-normal distribution with no significant deviations (mean = 1.78 ± 0.07, SD = 0.41 ± 0.05, n = 36). Based on this distribution, the probabilities of observing the three highest mean $[S]_{leaf}$ were P=0.008, 0.0015 and 0.0019 respectively. In general, plant species with greater mean $[Se]_{leaf}$ also had greater mean $[S]_{leaf}$, and the leaf Se/S quotient for most plant species was similar (Fig. 3C). However, although Se-accumulator plants had extraordinarily high mean $[Se]_{leaf}$, this was not reflected in their mean $[S]_{leaf}$ and there appeared to be a limit to $[S]_{leaf}$ of about 20 mg g^{-1} DW (Fig. 3). This is consistent with the observations of Hurd-Karrer (1937) and Rosenfeld and Beath (1964). Consequently, the leaf Se/S quotients (mg Se g^{-1} S) in Se-accumulator species were greater than those of other angiosperm species (Fig. 3), and there was little probability that the Se/S quotients of *Stanleya pinnata* ($P<0.0001$) or *Astragalus racemosus* ($P<0.0001$) leaves belonged to the normal distribution of leaf Se/S quotients shown by the other 37 angiosperm species studied (Fig. 2; mean = 1.72 ± 0.04, SD = 0.237 ± 0.028, n = 37). These observations are consistent not only with the strong correlation between shoot Se and S concentrations amongst angiosperm species grown under the same conditions when the analysis is restricted to Se-nonaccumulator crop plants (e.g. Hurd-Karrer, 1937; Bañuelos *et al*., 2005) but also with the lack of correlation between shoot Se and S concentrations when the analysis is broadened to include Se-accumulator plants (Rosenfeld and Beath, 1964; Feist and Parker, 2001). They indicate the occurrence of at least two distinct phenotypes for leaf Se/S quotient within the angiosperms. Interestingly, the mean leaf Se/S quotient for the 37 non-accumulator plants (1.72 mg Se g^{-1} S) approximated the Se/S quotient in

the mineral nutrient solution (1.71 mg Se g^{-1} S), suggesting no selectivity in the accumulation of S over Se in leaves of most angiosperm species.

It is noteworthy that all Brassicales species had high mean $[Se]_{leaf}$ and mean $[S]_{leaf}$ (Table 1), which is consistent with their unique S metabolism (Mengel and Kirkby, 2001; Willey and Wilkins, 2006) and extensive screens of angiosperms for phytoremediation of Se-laden soils, which has identified various *Brassica* species, such as Indian mustard (*Brassica juncea*), canola (*B. napus*) and broccoli (*B. oleracea*) as being particularly useful (Bañuelos *et al*., 1997, 2003, 2005; Terry *et al*., 2000; Wu, 2004; Bañuelos, 2002, 2006; Bañuelos and Lin, 2005). These species not only tolerate high tissue Se concentrations and accumulate biomass rapidly, but can also volatilise Se (Zayed *et al*., 1998; Terry *et al*., 2000; Bañuelos *et al*., 2005).

Ecological studies suggest that Se-accumulator plants include members of the Brassicaceae (*Stanleya pinnata*), Fabaceae (*Astragalus bisulcatus*, *A. racemosus*) and Asteraceae (*Aster occidentalis*, *Machaeranthera ramosa*). In the present study, *Stanleya pinnata* and *Astragalus racemosus* had significantly greater mean $[Se]_{leaf}$, mean $[S]_{leaf}$ and leaf Se/S quotients than other angiosperms (Table 1). The mean $[Se]_{leaf}$ and leaf Se/S quotient were lower in *Stanleya pinnata* than *Astragalus racemosus*, which is consistent with studies comparing the $[Se]_{leaf}$ of *Stanleya pinnata* with *Astragalus* species that hyperaccumulate Se (Goodson *et al*., 2003; Freeman *et al*., 2006; Galeas *et al*., 2007). Among the other Brassicaceae assayed, only *Brassica oleracea* had an abnormally high mean $[Se]_{leaf}$ and mean $[S]_{leaf}$, but this species had a leaf Se/S quotient similar to other angiosperms (1.51 ± 0.014 mg Se g^{-1} S, n = 6). These traits are shared with other members of the *Brassica* genus that can accumulate high $[Se]_{leaf}$ and $[S]_{leaf}$, but generally

have leaf Se/S quotients lower than those in the rhizosphere solution (Bell *et al*., 1992; Feist and Parker, 2001; Ellis and Salt, 2003). This suggests that the trait of a high leaf Se/S quotient evolved within the Brassicaceae family. Neither *Astragalus glycyphyllos* nor *A. sinicus* had greater mean $[Se]_{leaf}$, mean $[S]_{leaf}$ or leaf Se/S quotients than other angiosperms (Table 1). These observations are consistent with previous studies of Se accumulation in *Astragalus* species (Rosenfeld and Beath, 1964; Shrift, 1969; Davis, 1972, 1986; Sors *et al*., 2005*a*) and suggest that the trait of Se accumulation evolved independently within this genus also. Neither *Aster occidentalis* nor *Machaeranthera ramosa* were assayed in the present study, but neither *Machaeranthera bigelovii* nor *Machaeranthera tanacetifolia* showed abnormally high mean $[Se]_{leaf}$, mean $[S]_{leaf}$ or leaf Se/S quotients. This suggests that the trait of Se accumulation evolved within the *Machaeranthera* genus and is consistent with previous observations (Rosenfeld and Beath, 1964). Taken together, these data support the hypothesis that the trait of Se accumulation evolved by convergent evolution of appropriate biochemical pathways in disparate angiosperm clades (Brown and Shrift, 1982).

It is most likely that the distinct leaf Se/S quotients found in Se-accumulator and non-accumulator plants result from differences in the selectivity of Se and S delivery to the xylem in the root (White *et al*., 2004; Sors *et al*., 2005*b*; Broadley *et al*., 2006), although selective redistribution of organic Se and S compounds from the shoot to the root via the phloem might also be envisaged (Bell *et al*., 1992). It is unlikely that the distinct leaf Se/S quotients of Se-accumulator and non-accumulator plants result from differences in Se volatilization since, although species differ in their ability to volatilize both Se and S (Zayed *et al*., 1998; Terry *et al*., 2000; Bañuelos *et al*., 2005), even Indian

mustard, which volatilises more Se than most plants, volatilises amounts equivalent to only about 2% of the Se accumulated in shoots when grown hydroponically in a solution containing 20 μM Se (Terry *et al.*, 2000). Assuming that Se and S move symplastically across the root and that selenate and sulphate are loaded into the xylem, then the leaf Se/S quotient can be influenced by these processes of root cells: (1) selective uptake of selenate or sulphate across the plasma membrane, (2) selective metabolism of selenate or sulphate into other chemical forms, (3) selective sequestration of Se or S metabolites in the vacuole or (4) selective efflux of selenate or sulphate across the plasma membrane into the xylem. These processes can be differentiated on the basis of their predicted effects on root Se and S accumulation. Assuming that there is fixed Se:S stoichiometry for uptake across the plasma membrane, which reflects the Se/S quotient in the rhizosphere solution, an abnormally high Se/S quotient in roots of Se-accumulator plants would indicate that the selective uptake of selenate across the plasma membrane of root cells determined their high leaf Se/S quotient, whereas, if the Se/S quotient in roots of Se-accumulator plants was abnormally low, then the process impacting most on leaf Se/S quotient would not be the selective uptake of selenate by root cells. Since the Se/S quotients in roots of Se-accumulator plants are not abnormally low, and the percentage translocation of Se and S from roots to shoots appears similar across taxa (Bell *et al.*, 1992), it can be concluded that differences in the selectivity of uptake of selenate and sulphate underlie differences in the leaf Se/S quotient of Se-accumulator and non-accumulator plants. This conclusion is consistent with the hypothesis that the dominant HASTs of Se-accumulator plants are selective for selenate, whereas those in other angiosperm species are selective for sulphate (White *et al.*, 2004; Sors *et al.*, 2005*b*;

Broadley *et al*., 2006). A comparison of the protein structure of HASTs present in the plasma membrane of epidermal and/or cortical root cells of Se-accumulator and non-accumulator plants would allow the molecular basis of anion selectivity of HASTs to be determined. Thereafter, appropriate allelic variation in the domain(s) conferring selenate/sulphate selectivity, combined with transcriptional control of HAST expression, could be used to produce crops with increased $[Se]_{shoot}$ and leaf Se/S quotients plants through either conventional or GM approaches.

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FIGURE LEGENDS

Figure 1. Leaf Se concentrations of 36 angiosperm species grown hydroponically in a complete mineral nutrient solution containing 910 μM sulphate and 0.63 μM selenate, ranked according to their relative shoot Se concentrations estimated from a meta-analysis of literature data (White *et al*., 2004). The species numbers are: (1) *Sorghum bicolor*, (5) *Bromopsis inermis*, (8) *Panicum miliaceum*, (14) *Atriplex hortensis*, (19) *Trifolium subterraneum*, (23) *Lolium multiflorum*, (30) *Dactylis glomerata*, (34) *Lycopersicon pennellii*, (40) *Medicago sativa*, (43) *Medicago lupulina*, (50) *Trifolium pratense*, (56) *Bouteloua gracilis*, (60) *Solanum tuberosum*, (65) *Hordeum vulgare*, (70) *Holcus lanata*, (80) *Cynodon dactylon*, (84) *Sinapis alba*, (86) *Beta vulgaris*, (93) *Astragalus sinicus*, (104) *Astragalus glycyphyllos*, (112) *Agrostis stolonifera*, (123) *Sporobolus airoides*, (135) *Solanum melongena*, (142) *Raphanus sativa*, (153) *Cucumis sativa*, (163*) Brassica oleracea*, (168) *Helianthus annuus*, (169) *Oryzopsis hymenoides*, (171) *Linum usitatissimum*, (172) *Brassica juncea*, (174) *Oryza sativa*, (175) *Stanleya pinnata*, (176) *Brassica nigra*, (179) *Trifolium repens*, (180) *Brassica carinata*, (181) *Astragalus racemosus*.

Figure 2. Frequency distributions of (A) the natural log of leaf Se concentrations (mg Se kg^{-1} DW), (B) the natural log of leaf S concentrations (g Se kg^{-1} DW) and (C) leaf Se/S quotients (mg Se g^{-1} S) in 39 angiosperm species grown hydroponically in a complete mineral nutrient solution containing 910 μM sulphate and 0.63 μM selenate. Lines indicate the log-normal (A, mean = 2.35; SD = 0.496, n = 37; B, mean = 1.87, SD =

0.520, n = 39) or normal (C, mean = 1.72, SD = 0.240, n = 37) distributions fitted to data from either all species or the 37 species with the lowest leaf Se concentrations.

Figure 3. (A) The relationship between leaf Se concentration and leaf S concentration in 39 angiosperm species, including two species with extreme leaf Se concentration, grown hydroponically in a complete mineral nutrient solution containing 910 μM sulphate and 0.63 μM selenate. The line indicates a leaf S concentration of 20 g kg^{-1} DW. (B) The relationship between leaf Se concentration and leaf Se/S quotient in these 39 angiosperm species. The line indicates the Se/S in the nutrient solution supplied to the plants (1.71 mg S g^{-1} Se). (C) The relationships between leaf Se concentration and leaf S concentration (closed circles) or leaf Se/S quotient (open circles) in a subset of 37 of these species. The horizontal line indicates the Se/S quotient in the nutrient solution supplied to the plants. The sloping line indicates the regression between leaf Se concentration (mg kg^{-1} DW) and leaf S concentration (% DW) in these 37 species ($y = 0.0567x + 0.053$, $R^2 = 0.946$).

Table 1. Phylogenetic classification, durations of growth in hydroponics prior to harvest, dry weights (DW) of leaf tissue at harvest, dry weight / fresh weight (DW/FW) quotients of harvested leaves, leaf selenium concentrations, leaf sulphur concentrations and leaf Se/S quotients (expressed as mg Se g^{-1} S) of 39 angiosperm species supplied with a complete mineral nutrient solution containing 910 µM sulphate and 0.63 µM selenate. Species were ranked according to their relative shoot Se concentrations estimated from a meta-analysis of literature data (White *et al.*, 2004). (SE=standard error of the mean; n=number of samples). Three species with extreme shoot Se or S concentrations are indicated in bold type.

Rank	Species	Order	Growth Period (d)	Leaf DW (g) mean	Leaf DW (g) SE	Leaf DW/FW (%) mean	Leaf DW/FW (%) SE	Leaf Se (mg kg^{-1} DW) mean	Leaf Se (mg kg^{-1} DW) SE	Leaf S (g kg^{-1} DW) mean	Leaf S (g kg^{-1} DW) SE	Se/S (mg Se g^{-1} S) mean	Se/S (mg Se g^{-1} S) SE	n
1	*Sorghum bicolor*	Poales	24	1.47	0.16	9.71	0.30	7.1	0.53	4.2	0.08	1.67	0.10	6
5	*Bromopsis inermis*	Poales	35	1.48		13.49		12.7		6.6		1.91		1
8	*Panicum miliaceum*	Poales	28	1.85	0.25	8.48	0.12	11.9	0.23	5.4	0.18	2.21	0.10	6
14	*Atriplex hortensis*	Caryophyllales	27	0.96	0.37	8.69	0.08	6.5	0.28	4.6	0.07	1.41	0.08	3
19	*Trifolium subterraneum*	Fabales	27	0.36	0.03	17.25	0.53	14.6	0.33	8.3	0.12	1.76	0.05	6
23	*Lolium multiflorum*	Poales	28	1.85	0.11	12.75	0.36	7.5	0.28	4.5	0.11	1.65	0.03	6
30	*Dactylis glomerata*	Poales	28	0.86	0.19	16.18	0.22	7.1	0.50	3.9	0.12	1.80	0.09	6
34	*Lycopersicon pennellii*	Solanales	28	0.45	0.13	12.32	0.09	21.2	0.72	12.0	0.44	1.77	0.03	6
40	*Medicago sativa*	Fabales	27	0.08	0.08	16.81	0.88	6.0	0.60	4.2	0.23	1.41	0.07	6
43	*Medicago lupulina*	Fabales	27	0.47	0.04	16.62	0.66	5.7	0.30	3.5	0.06	1.64	0.07	6
50	*Trifolium pratense*	Fabales	27	0.50	0.05	14.98	0.97	6.4	0.34	4.2	0.28	1.53	0.09	6
56	*Bouteloua gracilis*	Poales	57	0.72	0.21	25.66	0.11	6.9	0.64	3.7	0.17	1.87	0.10	6
60	*Solanum tuberosum*	Solanales	27	1.22	0.21	8.66	0.82	9.8	0.48	4.8	0.08	2.02	0.00	6
65	*Hordeum vulgare*	Poales	30	1.09	0.47	9.83	0.77	12.3	0.79	7.1	0.47	1.73	0.03	5
70	*Holcus lanata*	Poales	28	0.75	0.11	13.25	0.92	8.7	0.54	5.1	0.32	1.70	0.06	6
80	*Cynodon dactylon*	Poales	35	2.40	0.28	13.84	0.11	14.1	0.54	7.2	0.39	1.97	0.05	4
84	*Sinapis alba*	Brassicales	27	0.52	0.62	11.60	0.82	21.9	0.67	12.9	0.36	1.70	0.03	6
86	*Beta vulgaris*	Caryophyllales	33	0.94	0.20	9.83	0.33	5.6	0.30	4.1	0.16	1.36	0.06	6
93	*Astragalus sinicus*	Fabales	35	0.45	0.29	14.37	0.55	6.9	1.00	4.1	0.30	1.68	0.02	2
10 4	*Astragalus glycyphyllos*	Fabales	26	0.42	0.17	18.48	0.22	7.0	1.47	6.3	0.26	1.12	0.04	6
11 2	*Agrostis stolonifera*	Poales	28	0.37	0.07	13.57	0.55	13.8	0.59	7.3	0.25	1.90	0.04	6
12 3	*Sporobolus airoides*	Poales	42	0.47	0.07	16.91	0.55	8.8	0.95	5.4	0.25	1.60	0.02	6
13 5	*Solanum melongena*	Solanales	35	0.42	0.20	9.08	0.55	5.8	0.08	3.3	0.06	1.80	0.05	6
14 2	*Raphanus sativa*	Brassicales	27	0.45	0.42	8.06	0.33	22.2	0.62	11.6	0.39	1.93	0.04	6
15 3	*Cucumis sativa*	Cucurbitales	23	0.31	0.07	10.76	0.44	10.4	0.49	6.5	0.31	1.62	0.03	6
16 3	***Brassica oleracea***	Brassicales	28	0.76	0.16	8.53	0.11	33.0	0.75	21.8	0.40	1.51	0.01	6
16 8	*Helianthus annuus*	Asterales	22	0.45	0.50	11.29	0.33	7.6	0.30	4.7	0.18	1.61	0.03	5
16 9	*Oryzopsis hymenoides*	Poales	52	0.25		25.41		5.5		3.6		1.53		1

171	*Linum usitatissimum*	Malpighiales	27	1.17	0.04	14.09	0.37	13.4	0.92	7.1	0.45	1.90	0.02	6
172	*Brassica juncea*	Brassicales	30	0.91	0.19	11.24	0.73	21.0	2.68	13.0	1.75	1.63	0.03	3
174	*Oryza sativa*	Poales	42	1.60		18.61		11.3		5.3		2.12		1
175	***Stanleya pinnata***	Brassicales	28	0.13	0.03	12.76	0.37	68.6	12.70	20.1	1.46	3.27	0.46	6
176	*Brassica nigra*	Brassicales	27	3.69	0.25	9.36	0.41	17.9	0.89	8.4	1.04	2.37	0.42	6
179	*Trifolium repens*	Fabales	27	0.41	0.08	11.16	0.47	7.5	0.48	4.7	0.23	1.62	0.07	6
180	*Brassica carinata*	Brassicales	27	6.97	0.85	9.85	0.19	17.0	0.29	9.9	0.14	1.72	0.02	6
181	***Astragalus racemosus***	Fabales	52	0.07	0.02	21.87	1.56	282.8	48.48	19.5	0.88	14.14	2.29	6
-	*Brassica arvense*	Brassicales	27	3.55	0.40	8.07	0.11	24.4	0.89	14.0	0.60	1.75	0.03	6
-	*Machaeranthera bigelovii*	Asterales	36	0.30	0.07	15.34	0.63	5.7	0.46	3.8	0.17	1.51	0.11	6
-	*Machaeranthera tanacetifolia*	Asterales	28	0.25	0.05	12.37	0.78	15.2	0.86	8.5	0.47	1.81	0.14	5

Figure 1

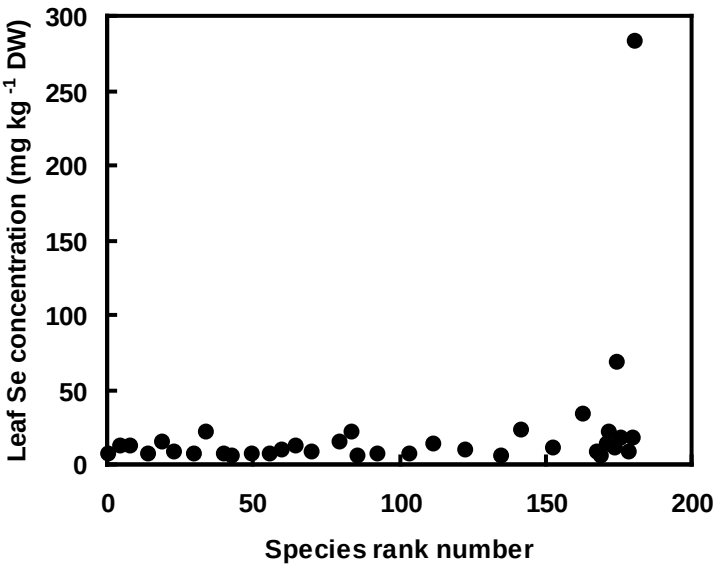

Leaf Se concentration (mg kg -1 DW)
300
250
200
150
100
50
0
0
50
100
150
200
Species rank number

Figure 2

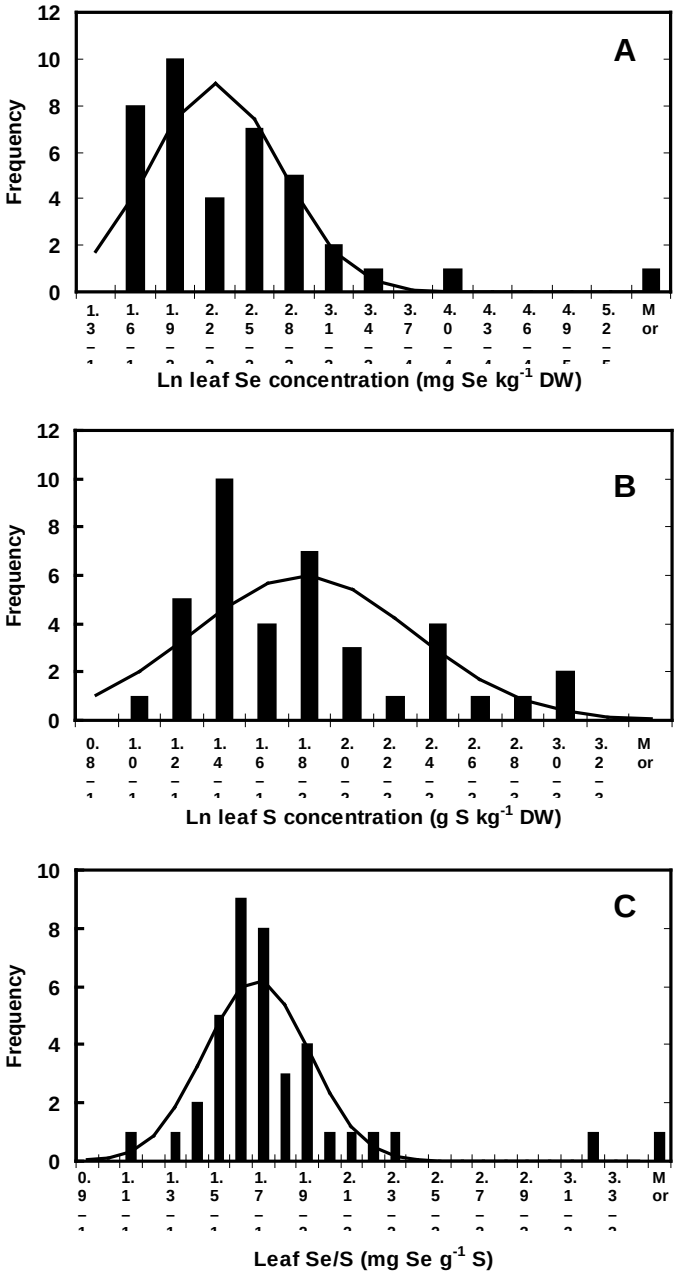
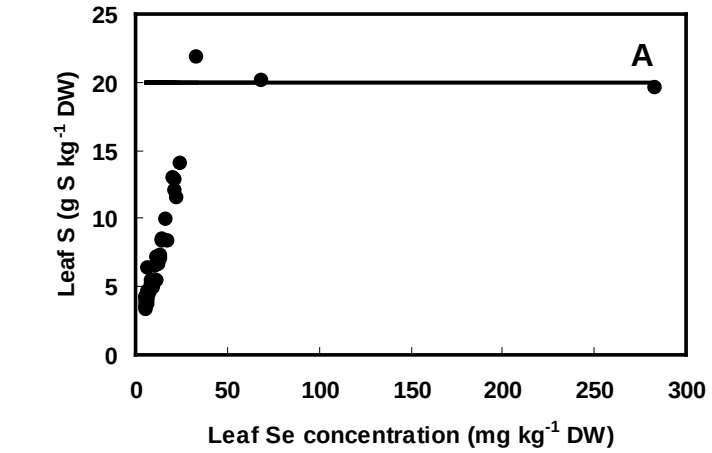
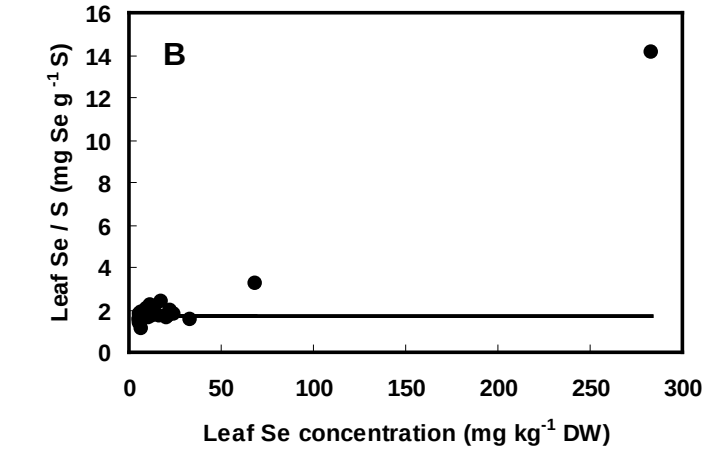
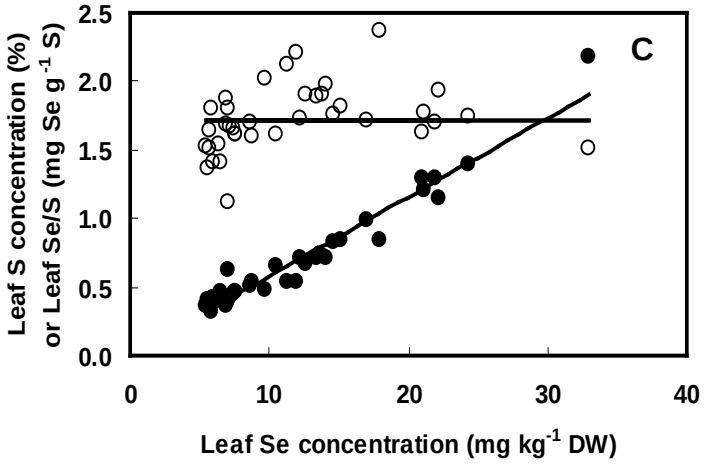

A
Frequency
Ln leaf Se concentration (mg Se kg-1 DW)
B
Frequency
Ln leaf S concentration (g S kg-1 DW)
C
Frequency
Leaf Se/S (mg Se g-1 S)

Figure3


A
Leaf S (g S kg-1 DW)
Leaf Se concentration (mg kg-1 DW)
0
5
10
15
20
25
0
50
100
150
200
250
300


B
Leaf Se / S (mg Se g -1 S)
Leaf Se concentration (mg kg-1 DW)
0
2
4
6
8
10
12
14
16
0
50
100
150
200
250
300


C
Leaf S concentration (%)
or Leaf Se/S (mg Se g -1 S)
Leaf Se concentration (mg kg-1 DW)
0.0
0.5
1.0
1.5
2.0
2.5
0
10
20
30
40

1