Selenium in higher plants: understanding mechanisms for biofortification and phytoremediation

Yong-Guan Zhu^{1,2}, Elizabeth A.H. Pilon-Smits³, Fang-Jie Zhao⁴, Paul N. Williams^{1,2} and Andrew A. Meharg⁵

¹ Research Center for Eco-environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

² Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China

³ Biology Department, Colorado State University, Fort Collins, CO 80523, USA

⁴ Soil Science Department, Rothamsted Research, Harpenden, AL5 2JQ, UK

⁵ Institute of Biological and Environmental Sciences, University of Aberdeen, Cruickshank Building, St Machar Drive, Aberdeen, AB24 3UU, UK

Selenium (Se) is an essential micronutrient for many organisms, including plants, animals and humans. As plants are the main source of dietary Se, plant Se metabolism is therefore important for Se nutrition of humans and other animals. However, the concentration of Se in plant foods varies between areas, and too much Se can lead to toxicity. As we discuss here, plant Se uptake and metabolism can be exploited for the purposes of developing high-Se crop cultivars and for plant-mediated removal of excess Se from soil or water. Here, we review key developments in the current understanding of Se in higher plants. We also discuss recent advances in the genetic engineering of Se metabolism, particularly for biofortification and phytoremediation of Se-contaminated environments.

Selenium in the environment

The metalloid selenium (Se) is ubiquitous in the environment, and its concentration in most soils ranges from 0.01 to 2.0 mg kg⁻¹, with a mean of ~0.4 mg kg⁻¹; however, higher concentrations (>10 mg kg⁻¹) can occur in seleniferous areas [1]. Soil Se concentration and bioavailability vary with parent material and environmental conditions, and the distribution of Se in soils is usually heterogeneous and site-specific [2]. However, Se bioavailability to plants can vary substantially for reasons that are still poorly understood.

Worldwide, interest in the biological impacts of Se on the environment and food chains is increasing because it is an essential micronutrient for many organisms, including humans and other animals (although it is toxic at higher concentrations) [3]. It is also a beneficial nutrient for many plants, including some higher plant taxa [4,5]. For humans and animals, the window between Se deficiency and toxicity is narrow, and both are observed in many areas of the world, sometimes in close proximity, such as in the Enshi district in China [6].

In organisms that require Se, selenocysteine (SeCys) is an essential component in so-called selenoproteins or

selenoenzymes [7], 25 of which have been identified in humans [8,9]. Selenoproteins have a redox function involved in free-radical scavenging [9], and several studies have shown that improving Se status can lower the risk of cancer [10–12]. As an exception, it was recently reported that in a prevention trial conducted in the USA on healthy men, the oral administration of Se at 200 mg day⁻¹ did not prevent prostate cancer, possibly because in this study the control group might have already received sufficient Se from their natural diet [13]. Nevertheless, for most people worldwide, as well as for livestock, plants are the main source of dietary Se [14]; thus, plant Se metabolism is of great importance for the Se nutrition of humans and animals.

Se concentrations in plant foods, such as rice (*Oryza sativa*) and wheat (*Triticum aestivum*), can vary greatly between countries and regions (Figure 1); thus, to avoid Se deficiency and toxicity, it is important to monitor and optimize crop Se concentrations. In a recent global survey of Se in rice purchased from retail outlets [15], it was highlighted that Se levels in major rice-producing and -consuming countries, such as Egypt, China and Thailand, are low, whereas they were higher in rice from the USA and India. The concentration of Se in wheat also shows large regional variation [16]. Where both rice and wheat are produced (e.g. India, China and Egypt), the Se concentrations of wheat and rice tend to be similar (Figure 1). Offsetting regions with inadequate Se by sourcing Se-rich grain is a practical solution to curtail the problem, but further characterization of both rice and wheat grain Se concentrations is needed [15].

Despite the widespread occurrence of Se deficiency globally, Se toxicity (selenosis) is a problem in some areas. Some soils and mineral deposits are naturally Se rich, and exploitation of these seleniferous soils or fossil fuels can lead to toxic accumulation of Se in the environment. Se contamination of sediments, soils and drainage water particularly occurs in arid seleniferous areas with intensive crop irrigation [17]. Perhaps the best example of elevated Se levels caused by local geology is the district of Enshi in Hubei Province, China [6,18]. Reported Se

Corresponding author: Zhu, Y.-G. (ygzhu@rcees.ac.cn).

Figure 1. Global comparison of Se levels in wheat (a) and rice grains (b). Mean average grain selenium concentrations are presented for key wheat- and rice-producing countries. Data from Refs [13,40,74–81].

toxicity in animals and humans is common in such Se-rich environments [18].

As a potential step towards finding a solution for elevated Se in the environment, as well as nutritional Se deficiency, better knowledge of Se metabolism in higher plants is crucial. Plant uptake and metabolism of Se can be exploited for the purposes of genetic biofortification (i.e. the development of high-Se crop cultivars combined with Se fertilization) and phytoremediation (plant-mediated removal of excess Se from soil or water). It is for these reasons that there is a resurgence of interest in Se in higher plants and, during the past few years, significant progress has been made in this area. Here, we do not intend to give a comprehensive account of the current knowledge of Se in higher plants; instead, we highlight the key developments made during the past five years or so, with the aim of identifying new research directions.

Plant uptake of selenium

Selenate is the predominant form of Se in alkaline and well-oxidized soils ($p_e + p_H > 15$), whereas in well-drained mineral soils with pH from acidic to neutral ($7.5 < p_e + p_H < 15$), Se exists predominantly as selenite [19]. Under strongly reduced soil conditions ($p_e + p_H < 7.5$), selenide becomes the dominant form. [19]. Plant uptake of selenate can be mediated by sulfate transporters owing to the chemical similarity between selenate and sulfate [20]. The selectivity of these transporters for selenate and sulfate varies between plant species and with nutritional status [21]. It has been suggested that the selectivity of the transport pathway for sulfate over selenate is lower at higher external sulfate concentrations and that the inducible sulfate transporters have higher selectivity for sulfate over selenate than do the constitutively active sulfate transporters [21]. Different sulfate transporters in a single plant might have different selectivity for sulfate versus selenate. *Arabidopsis thaliana* plants lacking SULTR1;2 but not SULTR1 show significantly enhanced selenate resistance [22]. In *Arabidopsis* mutants defective

in these two sulfate transporters, unequal functional redundancy was found with respect to selenate uptake and tolerance [23]. These studies suggest that SULTR1;2 is the predominant transporter for influx of selenate into the plant root. However, it is not known whether there is an increased selectivity of SULTR1;2 for selenate versus sulfate. A better understanding of the selectivity of different sulfate transporters at the molecular level might help the development of high- or low-Se plants through genetic engineering. It will be particularly interesting to study the properties of sulfate transporter homologues of Se hyperaccumulator plants. These plants occur only on seleniferous soils and typically contain tissue Se concentrations that are two orders of magnitude higher than the surrounding vegetation, up to 1% of dry weight [3]. Hyperaccumulators are characterized by a high leaf Se concentration, a higher Se:sulfur (S) ratio and a higher shoot:root Se concentration ratio [24,25], indicating altered regulation of sulfate and/or selenate transporters and the presence of (more) specific selenate transporters.

Less is known about the mechanisms of plant uptake of selenite, which might be prevalent in acidic to neutral soils or under reduced soil conditions, such as paddy soils. It has been suggested that the mechanism of selenite uptake by plant roots is not metabolically dependent [26]. However, recent results [27] show that selenite uptake by wheat was suppressed by the metabolic inhibitor carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP), inhibited by phosphate in the nutrient solution and enhanced by phosphorus (P) deficiency. It was argued that inconsistency in the rate of selenite uptake by plants could be ascribed to different phosphate concentrations present in the growth solutions used for different studies [28]. Thus, selenite uptake mechanisms require further investigation at both the physiological and molecular levels. In soil, selenite is less bioavailable to plants than is selenate because the former is more strongly adsorbed by iron oxides and/or hydroxides [29].

Translocation of Se from root to shoot depends on which Se species is supplied to the plant. In plants fed with selenate, Se is readily translocated to the shoot, and selenate is the predominant species in the xylem sap [27]. By contrast, in selenite-treated plants, most of the Se stays in the roots, with little selenite being detected in the xylem sap [27]. Selenite taken up by roots is readily converted to other forms, including selenomethionine (SeMet) and selenomethionine Se-oxide hydrate (SeOMet), but mostly into unidentified and water-insoluble forms [27]. Thus, in general, Se translocation from root to shoot is lower in plants fed with selenite than in those fed with selenate [26–28,30].

Selenium metabolism in plants

Owing to the chemical similarity of Se and S, selenate and selenite can be assimilated to SeCys and SeMet after plant uptake and non-specifically incorporated into any S compound [3]. The metabolic pathway of Se assimilation has been reviewed elsewhere [3,31], and it is not our intention to review the topic again here. Instead, we discuss the recent advances in genetic engineering of Se metabolism, particularly for the biofortification and phytoremediation of Se-contaminated environments.

Se speciation in plant tissues is important from the perspectives of understanding the metabolic pathways and human nutrition, and it has been investigated in several plant species. Se speciation varies with plant species and the form of Se fed to the plant [32–37]. For example, in Indian mustard (*Brassica juncea*), the main Se species is selenate when the plant is fed with selenate, whereas in plants fed with selenite, SeMet and SeOMet dominate [36]. Se-methylselenocysteine (SeMeSeCys) is also a major Se compound in Se-enriched garlic (*Allium sativum*), onion (*Allium cepa*), leek (*Allium ampeloprasum*) and broccoli (*Brassica oleracea*), accounting for approximately half of the total Se [38]. By contrast, SeMet is the predominant Se species in most grains, such as wheat (Figure 2), barley (*Hordeum vulgare*) and rye (*Secale cereale*), accounting for ~60–80% of the total Se [39]. However, the speciation might differ in grains with exceptionally high concentrations of Se. X-ray absorption near edge spectroscopy (XANES) analysis on one sample obtained from an Se-enriched area of Enshi district in South-Central China suggests that rice can also be comprised mainly of SeMeSeCys in addition to SeMet, when the total Se content of the endosperm is as high as 9 mg Se g⁻¹ [15]. However, in an earlier study that measured two samples of rice from Enshi fluorometrically, it was found that between 68% and 81% Se in rice grain was SeMet and no SeMeSeCys was detected, although the Se levels in the rice were much lower, with between 33% and 50% less Se [38]. As SeMeSeCys is the form of Se purported to confer the best anti-carcinogenic properties, this is advantageous from a human nutrition perspective. Nevertheless, both SeMeSeCys and SeMet confer additional health benefits over inorganic Se by either being more anti-carcinogenic or by being stored more effectively in the body [14,40]. If low-Se diets are to be successfully supplemented with appropriate Se-enriched foods, further characterization of Se speciation in staple foods from different parts of the world is necessary.

Figure 2. Representative Se speciation distribution in different plant tissues. (a) Rice (*Oryza sativa*) grain (endosperm (i) plus bran (ii)) characterized using X-ray analysis of near-edge spectra (XANES) [15]. (b) Spring wheat (*Triticum aestivum*) grain analyzed by LC-ICP-MS [39].

Genetics of Se accumulation in plants and its manipulation for biofortification

Numerous studies have reported inter- and intra-species differences in Se accumulation. Examples of interspecific variation in Se accumulation are Se hyperaccumulation in some plant species, such as two-grooved milkvetch (*Astragalus bisulcatus*) and prince's plume (*Stanleya pinnata*) [3,21,24]. Typical Se concentrations in field-grown hyperaccumulators can be 1000–10 000 mg kg⁻¹ dry weight, whereas in nonaccumulators, Se concentrations are usually <20 mg kg⁻¹ [25]. Moreover, hyperaccumulators show a pronounced and S-independent seasonal variation in Se concentration in different plant organs, suggestive of Se flow from roots to young leaves in early spring, from older to younger leaves and reproductive tissues in summer and from shoot to root in the fall [25]. Thus, whole-plant-level Se fluxes seem to be specialized in hyperaccumulators and separate from S movement. In another study, examination of Se accumulation by 16 populations of *S. pinnata*, an Se-hyperaccumulating species, found that shoot Se concentration can differ from 1.4 to 3.6-fold between populations [41]. Further studies revealed that both hyperaccumulators and nonaccumulators seem to access the same labile pools of Se in soil; therefore, Se-hyperaccumulators might be no better at accumulating Se from a relatively low-available-Se soil than are nonaccumulators, and root proliferation in Se-enriched soil (positive chemotaxis) might contribute to Se hyperaccumulation [42].

For crop plants, varietal differences in Se accumulation have also been reported. Surveys of Se concentrations in grain of ancestral and wild relatives of wheat, wheat landraces, and commercial cultivars grown in Australia and Mexico found no significant genotypic variation in grain Se among the modern wheat cultivars, but diploid wheat and rye had relatively higher grain Se concentrations [43]. The authors stated that soil Se bioavailability seems to be the predominant factor in determining grain Se, as was also reported recently in wheat [44]. Genotypic variation in Se concentration might become more prominent when the Se bioavailability in the medium is high. For example, two field studies conducted in the USA showed that there was significant difference in grain Se concentrations of 14 hard red winter wheat varieties, and that the varietal difference can be influenced by environmental factors [45].

Although it is well documented that inter- and intra-specific variation in Se accumulation in plants exists, the genetics of Se accumulation have rarely been investigated. Using recombinant inbred lines, the genetic basis of selenate tolerance of *A. thaliana* has recently been investigated. Three quantitative trait loci (QTLs) on chromosomes 1, 3 and 5 were found that could explain 24% of variation in tolerance index defined by root-length inhibition and 32% of the phenotypic variation in terms of root length [46]. Unfortunately, no published data exist on the QTLs associated with plant Se accumulation; such information would be useful for identifying specific genes responsible for Se accumulation and for developing high-Se cultivars through molecular breeding.

Although agronomic biofortification (i.e. increasing Se concentration in food through Se fertilization) has been proposed and practiced widely for various crops and countries [47–50], the inter- and intra-specific variation in plant Se concentrations could also be explored for bio-fortifying food to improve human Se nutrition [51]; this approach is generally termed ‘genetic biofortification’ [52]. Selecting existing cultivars for high Se in the edible parts of a given crop can have immediate impact on consumers’ dietary Se intake, and high-Se cultivars can be used in breeding programs to transfer the high-Se QTLs to high-yielding and locally adapted cultivars. Currently, there is no breeding program that specifically targets high-Se crop plants, perhaps because of the lack of high-Se crop cultivars.

The other reason for the lack of success in breeding high-Se crop cultivars might come from the fact that analyzing low concentrations of Se is not always achievable in laboratories, as inductively coupled plasma-mass spectrometry (ICP-MS) is required for detecting trace concentrations that are relevant to deficient environments. Therefore, it will be necessary to forge closer collaborations among plant breeders, molecular biologists and plant nutritionists and to combine high-throughput analysis of plant samples with breeding programs and genetic studies. This combination would be useful to dissect the genetics of Se uptake and partitioning in various plant species. Ionomics approaches to aid gene discovery have recently received increasing interest and help to marry genotype with phenotype [53]. A potential problem in Se-deficient areas, however, is that there is little available Se in soil, so genetic improvement would have little opportunity to increase grain Se content. To solve this problem, genetic fortification could be combined with agricultural fortification by application of a small amount of Se fertilizer [47,54].

Genetic engineering of selenium metabolism for phytoremediation

Studies so far on the genetic engineering of Se metabolism have focused on selenate reduction, prevention of SeCys incorporation into proteins and Se volatilization. Among one of the first attempts in genetic engineering to improve phytoremediation efficiency was the overexpression of ATP sulfurylase (APS1) in *B. juncea* to manipulate the initial step of Se metabolism (i.e. selenate reduction) [55]. Overexpression of APS1 led to increased selenate reduction *in planta* and resulted in a two–threefold increase in Se

accumulation in shoots and roots. The APS plants were also more tolerant to Se. Another study involved the overexpression of a mouse selenocysteine lyase (SL), an enzyme that specifically catalyzes the decomposition of SeCys into elemental Se and alanine, thus directing Se away from incorporation into proteins [56]. Overexpression of SL in *Arabidopsis* resulted in higher Se tolerance and up to a twofold enhanced Se accumulation. More recently, it was shown that overexpression of a plant homologue of the mouse SL, AtCpNifS, also significantly enhanced Se tolerance and accumulation [57]. In both the SL and CpNifS overexpressors, less Se was incorporated into protein, indicating that Se flow was successfully diverted toward less toxic forms of Se.

Another successful strategy for preventing incorporation of SeCys into protein has been to introduce a SeCys methyltransferase (SMT). SeMeSeCys is the predominant form of Se accumulated in hyperaccumulator plants [58,59]. The overexpression of SMT from the Se-hyperaccumulator *A. bisulcatus* in *Arabidopsis* and Indian mustard led to a significant increase in Se tolerance, accumulation and volatilization in both species [60,61].

A potential strategy for enhancing plant Se tolerance rather than accumulation is the upregulation of Se volatilization. Most plants can produce volatile dimethylselenide (DMeSe) from methionine, whereas hyperaccumulators produce dimethyldiselenide (DMeDSe) from SeMeSeCys. S-adenosyl-L-Met:L-Met S-methyltransferase (MMT) is the key enzyme responsible for the methylation of Se-Met to Se-methyl Se-Metionine (SeMeMet), which is the precursor of volatile DMeSe [62]. However, whether manipulation of SeMeMet can be effective in increasing Se volatilization has yet to be tested. In another genetic approach, DMeSe volatilization could be enhanced two–threefold by overexpression of cystathionine- γ -synthase, the key enzyme for the conversion of (Se-)cysteine to (Se-)methionine [63]. Further identification of different volatile Se compounds could aid in the dissection of Se metabolic pathways in plants of different species, as well as in transgenics [64].

Once transgenic approaches have shown promising results with respect to Se tolerance, accumulation or volatilization, they need to be tested in the field for their potential for phytoremediation or as biofortified foods. To this end, the first small-scale experiment using transgenic lines of Indian mustard overexpressing genes encoding the enzymes APS, γ -glutamyl-cysteine synthetase (ECS) and glutathione synthetase (GS) was performed [65,66]. The APS plants have been described above; the ECS and GS plants overproduce glutathione (GSH), an important antioxidant thiol that is also involved in metal tolerance. The transgenic plants performed well in the field both in terms of biomass production and Se accumulation, with up to 4.5-fold higher Se accumulation in APS transgenics compared with wild-type plants [65]. In a second field study, transgenic SL and SMT plants also showed promising results, with up to twofold higher Se accumulation than in wild-type plants [66]. These studies offer hope that, through genetic manipulation of high biomass, fast-growing plants, Se phytoremediation can be developed into a viable option, while producing crops with possibly better nutritional quality.

Perspectives and emerging interests

Se uptake and metabolism in plants are complex and are closely related to soil properties (i.e. Se speciation and its dynamics in soil). Key processes and points where genetic manipulation has been shown to enhance Se uptake, tolerance and/or metabolism are summarized in [Figure 3](#); mechanisms that have yet to be clarified are also indicated. From a human nutrition perspective, what seems to be most urgent is the elucidation of how and in what forms Se is loaded to the grain of staple foods, such as rice and wheat, and what manipulations can be adopted to improve the accumulation of favorable Se compounds in these grains. In addition to these issues directly related to Se uptake and metabolism, there are emerging interests in Se in the food chain, which can be highlighted as follows.

Selenium mitigation of the health risk of arsenic accumulation in rice

Some toxicological studies seem to show that Se is an antagonist to arsenic (As) toxicity and carcinogenicity in mammalian models [67]. It is not clear whether this is due to: (i) the free-radical scavenging nature of Se compounds; (ii) Se directly affecting As metabolism, perhaps through altering As methylation [68]; or through the direct formation of As–Se compounds, such as seleno-bis (S-glutathionyl) arsinium [69]; or (iii) excretion patterns [70].

Genetic engineering for biofortification

Genetic engineering for biofortification is, in many ways, the same as for phytoremediation: higher Se levels in harvestable plant parts are good for both. An important

Figure 3. Overview of Se metabolism and partitioning in plants, with an emphasis on genetic engineering approaches that have been shown to modify these processes. Enzymes in the yellow circles are those known to promote the conversion after overexpression (or at those points where Se uptake and metabolism can be manipulated); arrows leaving cells indicate the translocation of Se within and from the plant; dashed arrows indicate that the process is not yet confirmed. Abbreviations: PT, high-affinity phosphate transporters; Secysth, Se-systhionine; Sehocys, Se-homocysteine; ST, high-affinity sulfate transporters. Purple box = unknown transport for organic Se; blue box = selenate efflux pump.

difference between genetic engineering for phytoremediation and biofortification purposes is that Se in tissues of edible plants should not reach concentrations that are toxic either to the plants themselves or to the animals that eat them. Another difference is that, for biofortification, it is preferable that specific forms of Se are accumulated, as some forms have more powerful anti-carcinogenic properties than do others. SeMeSeCys, for instance, is one of the best forms of Se to use in fortified foods and thus overexpression of SMT might be the best option for the purpose of biofortification. With the advancement of molecular biology, it might also be possible to overexpress targeted gene(s) in specific plant tissues, such as in the grain, or to overexpress these targeted genes so that anti-carcinogenic compounds can be easily extracted for mass production.

Unifying phytoremediation, bioenergy and the production of anticarcinogenic selenocompounds?

If plants are used to clean up soils or waters contaminated with just Se, the resulting plant material could be used as fortified foods. Studies are already underway to test the effects of feeding Se-rich canola meal to cows or sheep [71]. A third potential use of the Se-rich plant material is as biofuels. The use of biomass grown from phytoremediation of Se-laden effluent is also being used [72]. In the future, soils and wastewaters contaminated with Se could be cleaned up by Se-accumulating crops that are subsequently used as fortified food for livestock or as biofuels. Unifying phytoremediation and bioenergy might provide a sustainable option for the management of other contaminated areas, particularly those heavily impacted by mining activities, as the use of good-quality arable land for bioenergy production is deemed unsustainable owing to increasing global food demand [73]. It is envisaged that the issues discussed in this paper might also be applicable to other trace elements that are beneficial to human health but toxic at higher concentrations.

Acknowledgements

This study is partly supported by the Natural Science Foundation of China (20720102042) and the Chinese Academy of Sciences (KZCX1-YW-06-3).

References

- 1 Fordyce, F.M. (2005) Selenium deficiency and toxicity in the environment. In *Essentials of Medical Geology* (Selinus, O. et al., eds), pp. 373–415, Elsevier
- 2 Wang, Z.J. and Gao, Y.X. (2001) Biogeochemical cycling of selenium in Chinese environments. *Appl. Geochem.* 16, 1345–1351
- 3 Terry, N. et al. (2000) Selenium in higher plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 51, 401–432
- 4 Pilon-Smits, E.A.H. et al. (2009) Physiological functions of beneficial elements. *Curr. Opin. Plant Biol.* 10.1016/j.pbi.2009.04.009
- 5 Lyons, G. et al. (2009) Selenium increases seed production in *Brassica*. *Plant Soil* 318, 73–80
- 6 Fordyce, F.M. et al. (2000) Soil, grain and water chemistry in relation to human selenium-responsive diseases in Enshi District. *China. Appl. Geochem.* 15, 117–132
- 7 Rayman, M.P. (2002) The argument for increasing selenium intake. *Proc. Nutr. Soc.* 61, 203–215
- 8 Kryukov, G.V. et al. (2003) Characterization of mammalian selenoproteomes. *Science* 300, 1439–1443
- 9 Lu, J. and Holmgren, A. (2009) Selenoproteins. *J. Biol. Chem.* 284, 723–727
- 10 Clark, L.C. et al. (1996) Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin a randomized controlled trial – a randomized controlled trial. *J. Am. Med. Assoc.* 276, 1957–1963
- 11 Wallace, K. et al. (2009) Selenium and risk of bladder cancer: a population-based case-control study. *Cancer Prev. Res.* 2, 70–73
- 12 Reid, M.E. et al. (2008) The nutritional prevention of cancer: 400 mcg per day selenium treatment. *Nutr. Cancer* 60, 155–163
- 13 Lippman, S.M. et al. (2009) Effect of selenium and vitamin E on risk of prostate cancer and other cancers: The Selenium and Vitamin E Cancer Prevention Trial (SELECT). *J. Am. Med. Assoc.* 301, 39–51
- 14 Rayman, M.P. (2008) Food-chain selenium and human health: emphasis on intake. *Br. J. Nutr.* 100, 254–268
- 15 Williams, P.N. et al. (2009) Selenium characterisation in the global rice supply chain. *Environ. Sci. Technol.* 10.1021/es900671m
- 16 Hawkesford, M.J. and Zhao, F.J. (2007) Strategies for increasing the selenium content of wheat. *J. Cereal Sci.* 46, 282–292
- 17 Ohlendorf, H.M. et al. (1986) Selenium and heavy-metals in San-Francisco bay diving ducks. *J. Wildl. Manage.* 50, 64–70
- 18 Mao, D. et al. (1990) Endemic investigation of Se toxicity in E-xi District. *Chin. J. Epidemiol* 9, 311
- 19 Elrashidi, M.A. et al. (1987) Chemical-equilibria of selenium in soils – a theoretical development. *Soil Sci.* 144, 141–152
- 20 Anderson, J. (1993) Selenium interactions in sulfur metabolism. In *Sulfur Nutrition and Assimilation in Higher Plants: Regulatory Agricultural and Environmental Aspects* (De Kok, I.S. et al., eds), pp. 49–60, SPB Academic
- 21 White, P.J. et al. (2004) Interactions between selenium and sulphur nutrition in *Arabidopsis thaliana*. *J. Exp. Bot.* 55, 1927–1937
- 22 El Kassis, E. et al. (2007) Characterization of a selenate-resistant *Arabidopsis* mutant. Root growth as a potential target for selenate toxicity. *Plant Physiol.* 143, 1231–1241
- 23 Barberon, M. et al. (2008) Unequal functional redundancy between the two *Arabidopsis thaliana* high-affinity sulphate transporters SULTR1;1 and SULTR1;2. *New Phytol.* 180, 608–619
- 24 White, P.J. et al. (2007) Extraordinarily high leaf selenium to sulfur ratios define ‘Se-accumulator’ plants. *Ann. Bot. (Lond.)* 100, 111–118
- 25 Galeas, M.L. et al. (2007) Seasonal fluctuations of selenium and sulfur accumulation in selenium hyperaccumulators and related nonaccumulators. *New Phytol.* 173, 517–525
- 26 Arvy, M.P. (1993) Selenate and selenite uptake and translocation in bean-plants (*Phaseolus vulgaris*). *J. Exp. Bot.* 44, 1083–1087
- 27 Li, H.F. et al. (2008) Selenium uptake, translocation and speciation in wheat supplied with selenate or selenite. *New Phytol.* 178, 92–102
- 28 Hopper, J. and Parker, D.R. (1999) Plant availability of selenite and selenate as influenced by the competing ions phosphate and sulfate. *Plant Soil* 210, 199–207
- 29 Barrow, N.J. and Whelan, B.R. (1989) Testing a mechanistic model. VIII. The effects of time and temperature of incubation on the sorption and subsequent desorption of selenite and selenate by a soil. *J. Soil Sci.* 40, 29–37
- 30 de Souza, M.P. et al. (1998) Rate-limiting steps in selenium assimilation and volatilization by Indian mustard. *Plant Physiol.* 117, 1487–1494
- 31 Sors, T.G. et al. (2005) Selenium uptake, translocation, assimilation and metabolic fate in plants. *Photosynth. Res.* 86, 373–389
- 32 Ximenez-Embun, P. et al. (2004) Establishment of selenium uptake and species distribution in lupine, Indian mustard, and sunflower plants. *J. Agric. Food Chem.* 52, 832–838
- 33 Kapolna, E. and Fodor, P. (2006) Speciation analysis of selenium enriched green onions (*Allium fistulosum*) by HPLC-ICP-MS. *Microchem. J.* 84, 56–62
- 34 Kapolna, E. et al. (2007) Selenium speciation studies in Se-enriched chives (*Allium schoenoprasum*) by HPLC-ICP-MS. *Food Chem.* 101, 1398–1406
- 35 Grant, T.D. et al. (2004) Identification and characterization of Se-methyl selenomethionine in *Brassica juncea* roots. *J. Chromatogr. A* 1026, 159–166
- 36 Kahakachchi, C. et al. (2004) Chromatographic speciation of anionic and neutral selenium compounds in Se-accumulating *Brassica juncea* (Indian mustard) and in selenized yeast. *J. Chromatogr. A* 1054, 303–312
- 37 Wang, Z.J. et al. (1996) Distribution of Se in soybean samples with different Se concentration. *J. Agric. Food Chem.* 44, 2754–2759

- 38 Beilstein, M.A. *et al.* (1991) Chemical forms of selenium in corn and rice grown in a high selenium area of China. *Biomed. Environ. Sci.* 4, 392–398
- 39 Stadlober, M. *et al.* (2001) Effects of selenate supplemented fertilisation on the selenium level of cereals – identification and quantification of selenium compounds by HPLC-ICP-MS. *Food Chem.* 73, 357–366
- 40 Rayman, M.P. *et al.* (2008) Food-chain selenium and human health: spotlight on speciation. *Br. J. Nutr.* 100, 238–253
- 41 Feist, L.J. and Parker, D.R. (2001) Ecotypic variation in selenium accumulation among populations of *Stanleya pinnata*. *New Phytol.* 149, 61–69
- 42 Goodson, C.C. *et al.* (2003) Soil selenium uptake and root system development in plant taxa differing in Se-accumulating capability. *New Phytol.* 159, 391–401
- 43 Lyons, G. *et al.* (2005) Selenium concentration in wheat grain: Is there sufficient genotypic variation to use in breeding? *Plant Soil* 269, 369–380
- 44 Zhao, F.J. *et al.* (2009) Variation in mineral micronutrient concentrations in grain of wheat lines of diverse origin. *J. Cereal Sci.* 49, 290–295
- 45 Garvin, D.F. *et al.* (2006) Historical shifts in the seed mineral micronutrient concentration of US hard red winter wheat germplasm. *J. Sci. Food Agric.* 86, 2213–2220
- 46 Zhang, L.H. *et al.* (2006) Mapping quantitative trait loci associated with selenate tolerance in *Arabidopsis thaliana*. *New Phytol.* 170, 33–42
- 47 Fang, Y. *et al.* (2008) Effect of foliar application of zinc, selenium, and iron fertilizers on nutrients concentration and yield of rice grain in China. *J. Agric. Food Chem.* 56, 2079–2084
- 48 Cao, Z.H. *et al.* (2001) Selenium geochemistry of paddy soils in Yangtze River Delta. *Environ. Int.* 26, 335–339
- 49 Smrkolj, P. *et al.* (2006) Selenium species in buckwheat cultivated with foliar addition of Se(VI) and various levels of UV-B radiation. *Food Chem.* 96, 675–681
- 50 Walburger, K.J. *et al.* (2008) The effect of fertilizing forage with sodium selenate on selenium concentration of hay, drain water and serum selenium concentrations in beef heifers and calves. *Can. J. Anim. Sci.* 88, 79–83
- 51 Graham, R.D. *et al.* (2007) Nutritious subsistence food systems. *Adv. Agron.* 92, 1–74
- 52 Broadley, M.R. *et al.* (2006) Biofortification of UK food crops with selenium. *Proc. Nutr. Soc.* 65, 169–181
- 53 Salt, D.E. *et al.* (2008) Ionomics and the study of the plant ionome. *Annu. Rev. Plant Biol.* 59, 709–733
- 54 White, P.J. and Broadley, M.R. (2009) Biofortification of crops with seven mineral elements often lacking in human diets – iron, zinc, copper, calcium, magnesium, selenium and iodine. *New Phytol.* 182, 49–84
- 55 Pilon-Smits, E.A.H. *et al.* (1999) Selenium volatilization and accumulation by twenty aquatic plant species. *J. Environ. Qual.* 28, 1011–1018
- 56 Pilon, M. *et al.* (2003) Enhanced selenium tolerance and accumulation in transgenic *Arabidopsis* expressing a mouse selenocysteine lyase. *Plant Physiol.* 131, 1250–1257
- 57 Van Hoewyk, D. *et al.* (2005) Overexpression of AtCpNifS enhances selenium tolerance and accumulation in *Arabidopsis*. *Plant Physiol.* 139, 1518–1528
- 58 Neuhierl, B. and Bock, A. (1996) On the mechanism of selenium tolerance in selenium-accumulating plants – purification and characterization of a specific selenocysteine methyltransferase from cultured cells of *Astragalus bisulcatus*. *Eur. J. Biochem.* 239, 235–238
- 59 Freeman, J.L. *et al.* (2006) Spatial imaging, speciation, and quantification of selenium in the hyperaccumulator plants *Astragalus bisulcatus* and *Stanleya pinnata*. *Plant Physiol.* 142, 124–134
- 60 LeDuc, D.L. *et al.* (2004) Overexpression of selenocysteine methyltransferase in *Arabidopsis* and Indian mustard increases selenium tolerance and accumulation. *Plant Physiol.* 135, 377–383
- 61 LeDuc, D.L. *et al.* (2006) Overexpressing both ATP sulfurylase and selenocysteine methyltransferase enhances selenium phyto remediation traits in Indian mustard. *Environ. Pollut.* 144, 70–76
- 62 Tagmount, A. *et al.* (2002) An essential role of S-adenosyl-L-methionine: L-methionine S-methyltransferase in selenium volatilization by plants. Methylation of selenomethionine to selenium-methyl-L-selenium-methionine, the precursor of volatile selenium. *Plant Physiol.* 130, 847–856
- 63 Van Huysen, T. *et al.* (2003) Overexpression of cystathionine-gamma-synthase enhances selenium volatilization in *Brassica juncea*. *Planta* 218, 71–78
- 64 Kubachka, K.M. *et al.* (2007) Selenium volatiles as proxy to the metabolic pathways of selenium in genetically modified *Brassica juncea*. *Environ. Sci. Technol.* 41, 1863–1869
- 65 Banuelos, G. *et al.* (2005) Field trial of transgenic Indian mustard plants shows enhanced phyto remediation of selenium-contaminated sediment. *Environ. Sci. Technol.* 39, 1771–1777
- 66 Banuelos, G. *et al.* (2007) Transgenic Indian mustard overexpressing selenocysteine lyase or selenocysteine methyltransferase exhibit enhanced potential for selenium phyto remediation under field conditions. *Environ. Sci. Technol.* 41, 599–605
- 67 Kibriya, M.G. *et al.* (2007) Changes in gene expression profiles in response to selenium supplementation among individuals with arsenic-induced pre-malignant skin lesions. *Toxicol. Lett.* 169, 162–176
- 68 Styblo, M. and Thomas, D.J. (2001) Selenium modifies the metabolism and toxicity of arsenic in primary rat hepatocytes. *Toxicol. Appl. Pharmacol.* 172, 52–61
- 69 Burns, F.J. *et al.* (2008) Mechanism of selenium-induced inhibition of arsenic-enhanced UVR carcinogenesis in mice. *Environ. Health Perspect.* 116, 703–708
- 70 Miyazaki, K. *et al.* (2003) Negative correlation between urinary selenium and arsenic levels of the residents living in an arsenic-contaminated area in Bangladesh. *J. Health Sci.* 49, 239–242
- 71 Bañuelos, G.S. (2009) Phyto remediation of selenium-contaminated soil and water produces biofortified products and new agricultural byproducts. In *Biofortified Agricultural Products* (Bañuelos, G.S. and Lin, Z.Q., eds), pp. 57–70, CRC Press
- 72 Stapleton, J.J. and Banuelos, G.S. (2009) Biomass crops can be used for biological disinfection and remediation of soils and water. *Calif. Agric.* 63, 41–46
- 73 Searchinger, T. *et al.* (2008) Use of US croplands for biofuels increases greenhouse gases through emissions from land-use change. *Science* 319, 1238–1240
- 74 Gissel-Nielsen, G. *et al.* (1984) Selenium in soils and plants and its importance in livestock and human nutrition. *Adv. Agron.* 37, 397–460
- 75 Alamin, M.B. *et al.* (2006) Determination of mercury and selenium in consumed food items, in Libya using instrumental and radiochemical NAA. *J. Radioanal. Nucl. Chem.* 270, 143–146
- 76 Adams, M.L. *et al.* (2002) Evidence of low selenium concentrations in UK bread-making wheat grain. *J. Sci. Food Agric.* 82, 1160–1165
- 77 Pappa, E.C. *et al.* (2006) Selenium content in selected foods from the Greek market and estimation of the daily intake. *Sci. Total Environ.* 372, 100–108
- 78 Spadoni, M. *et al.* (2007) Bioaccessible selenium in Italian agricultural soils: comparison of the biogeochemical approach with a regression model based on geochemical and pedoclimatic variables. *Sci. Total Environ.* 376, 160–177
- 79 Wolnik, K.A. *et al.* (1983) Elements in major raw agricultural crops in the United States. 2. Other elements in lettuce, peanuts, potatoes, soybeans, sweet corn, and wheat. *J. Agric. Food Chem.* 31, 1244–1249
- 80 Boila, R.J. *et al.* (1993) The concentration of selenium in the grain from wheat, barley and oats grown at selected locations throughout Manitoba. *Can. J. Anim. Sci.* 73, 217–221
- 81 FAO and WHO (2001) *Human Vitamin and Mineral Requirements: Report of a Joint FAO/WHO Expert Consultation, Bangkok, Thailand*, Food and Nutrition Division, FAO Rome. (<ftp://ftp.fao.org/docrep/fao/004/y2809e/y2809e00.pdf>)