

## ***Part III***

# Selenium Biofortification

---



# Chapter 8

## Selenium hyperaccumulation in plants



*Leonardo Warzea Lima and Michela Schiavon*

### 8.1 INTRODUCTION

Selenium (Se) is a metal(loid) that functions as a structural component of essential selenoproteins serving a variety of metabolic functions in many living organisms, including humans and animals (Kieliszek, 2019). However, its essentiality to vascular plants has not yet been established (Schiavon & Pilon-Smits, 2017a). Edible plants represent a source of Se for human diet and their capacity to accumulate Se largely varies depending on phytoavailable soil Se, the plant species, and the existence of selective uptake mechanisms (White, 2016).

Tissue Se concentrations higher than 100 mg Se kg<sup>-1</sup> DW typically cause toxicity to most plants because Se amino acids formed during Se assimilation might be incorporated in proteins in place of their sulfur (S) analogs, hence leading to protein folding disturbance and function disruption (Van Hoewyk, 2013). Nevertheless, current literature supports a role for Se as an antioxidant in plants at low concentrations (1–10 mg Se kg<sup>-1</sup> DW) owing to its capacity to foster, either directly or indirectly, the activity of radical-scavenging enzymes and the synthesis of non-enzymatic antioxidant compounds (Chauhan *et al.*, 2019; Kamran *et al.*, 2019). Doing this, Se contrasts the generation of reactive oxygen species (ROS) in cells, which can injure a variety of biomolecules, especially DNA and membrane components (Natasha *et al.*, 2018). Other positive outcomes of low Se concentrations in plants include growth stimulation, reduction of toxic metal accumulation, retardation of leaf senescence, and protection from

pathogens and predators (Pilon-Smits, 2019; Schiavon & Pilon-Smits, 2017a; Valdez Barillas *et al.*, 2011).

Beneficial effects of Se have been described in several plant species, whether non-Se-hyperaccumulators or Se-hyperaccumulators. The latter, in particular, are native to seleniferous soils and can be potentially used in certain phytotechnologies, such as phytoremediation, biofortification, and agromining, by virtue of their extremely high capacity to accumulate Se without experiencing toxicity (Reeves *et al.*, 2018; Schiavon & Pilon-Smits, 2017b). Recent studies have highlighted several mechanisms that might explain why some plants developed the Se hyperaccumulation and hypertolerance traits, such as the existence of specific tissue Se sequestration patterns and the constitutive elevated expression of genes coding for Se/S transporters and Se/S assimilation enzymes, antioxidant enzymes, and proteins involved in hormone biosynthesis, signaling and interactions with ecological partners (Lima *et al.*, 2018; Wang *et al.*, 2018). It is believed that Se hyperaccumulation is the result of convergent evolution of Se transporters and specific or alternative biochemical pathways in different angiosperm clades, which occurred during geological periods when seleniferous soils were widely distributed (White, 2016). In support of this hypothesis are several pieces of evidence. Compared to non-hyperaccumulators, Se-hyperaccumulators have higher shoot/root Se ratios, Se/S ratios (indicative of more selective mechanisms for Se uptake and translocation over S), and higher Se translocation from sources to sinks (El Mehdawi *et al.*, 2018; Schiavon *et al.*, 2015). Also, they contain more organic Se, especially in the form of methylselenocysteine (SeMetCys), which suggests intense Se assimilation and the existence of biochemical mechanisms aimed at preventing misincorporation of Se-amino acids in proteins (White, 2018). SeMetCys primarily accumulates in epidermal cells and reproductive organs of these Se-hyperaccumulator plants (Pilon-Smits, 2019).

In this chapter, we aim to describe (i) differences in uptake, accumulation, and metabolic fate of Se between the non-Se accumulator and Se hyperaccumulator species, (ii) key factors responsible for the evolution of the Se hyperaccumulation and hypertolerance traits, and (iii) potential uses of Se-hyperaccumulators in agro- and phytotechnologies.

## 8.2 VARIATION IN Se ACCUMULATION BETWEEN HYPERACCUMULATORS AND NON-HYPERACCUMULATORS

### 8.2.1 Se uptake in plants

Despite Se accumulation by plants tightly relating to soil Se, angiosperm species can differ in their capacity to take up Se when growing in the same environment. This suggests that Se accumulation in plants also depends on the selectivity of the transport mechanisms used to acquire Se.

Although plants do not have an essential demand for Se, they can take up different inorganic and organic forms of this element, whose oxidation state in soils ranges between  $-2$  and  $+6$  (Hartikainen, 2005). Among inorganic Se species, selenate ( $\text{SeO}_4^{2-}$ ) is the most available to plants in oxic soils of high redox potential (Hartikainen, 2005). Selenate is the chemical (toxic) analog to sulfate, and thus plants make use of sulfate transporters to allow selenate to enter the root cells (El-Mehdawi *et al.*, 2018). In anoxic soils characterized by low redox potential and pH from neutral to acidic, like paddy soils, selenate tends to be converted to selenite ( $\text{SeO}_3^{2-}$ ), which is acquired by plants using either phosphate transporters, silicon transporters, or aquaporins, depending on the selenite species (selenite anion ( $\text{SeO}_3^{2-}$ ), hydrogenselenite ion ( $\text{HSeO}_3^-$ ), and selenous acid ( $\text{H}_2\text{SeO}_3$ )) (Shahid *et al.*, 2018). Plants can also take up elemental Se, but not selenide or colloidal elemental Se (Winkel *et al.*, 2015).

### 8.2.2 Se accumulators and hyperaccumulating plants

There is broad variation in the capacity of plants to accumulate Se in their organs, which is largely affected by soil Se content and phytoavailability (White, 2016, 2018). Plants thriving in soils either naturally rich in available Se or contaminated with Se due to anthropogenic activities or dust depositions in coal-burning areas are inclined to accumulate more Se than plants colonizing low Se areas (Schiavon and Pilon-Smits, 2017a, b; White, 2016). The Se content in soils is commonly below  $2 \mu\text{g g}^{-1}$  but can reach several hundred  $\mu\text{g g}^{-1}$  (up to  $1.2 \text{ mg g}^{-1}$ ) in soils derived from sedimentary rocks, especially Cretaceous sediments rich in selenites and selenides associated with sulfide minerals (Winkel *et al.*, 2015). These soils are termed seleniferous and are located in the Great Plains of the USA, Canada, Brazil, Australia, India, China, and Russia, and usually support a distinctive pattern of vegetation (Oldfield, 2002; Winkel *et al.*, 2015). Plants growing in seleniferous areas can exclude or actively remove Se from their tissues, or tolerate high internal Se concentrations (White, 2018). Despite such a different behavior towards Se, all these plants have a high Se concentration in their tissues and therefore must exhibit a minimal ability to tolerate elevated Se concentrations.

Based on the maximum Se concentration in their shoot, higher plants can be sorted into two major ecological groups, that is, Se non-hyperaccumulators and Se-hyperaccumulators. Se non-hyperaccumulators include non-accumulators and accumulator species. The majority of plants fall in the category of non-accumulators and are unable to accumulate and tolerate more than  $100 \mu\text{g Se g}^{-1}$  DW. Therefore, these plants cannot colonize seleniferous soils and, in most cases, their Se concentration is below  $1 \mu\text{g g}^{-1}$  DW (White, 2016, 2018). In contrast, Se-accumulators can tolerate tissue Se concentrations approaching  $1 \text{ mg Se g}^{-1}$  DW and can populate both non-seleniferous and seleniferous soils.

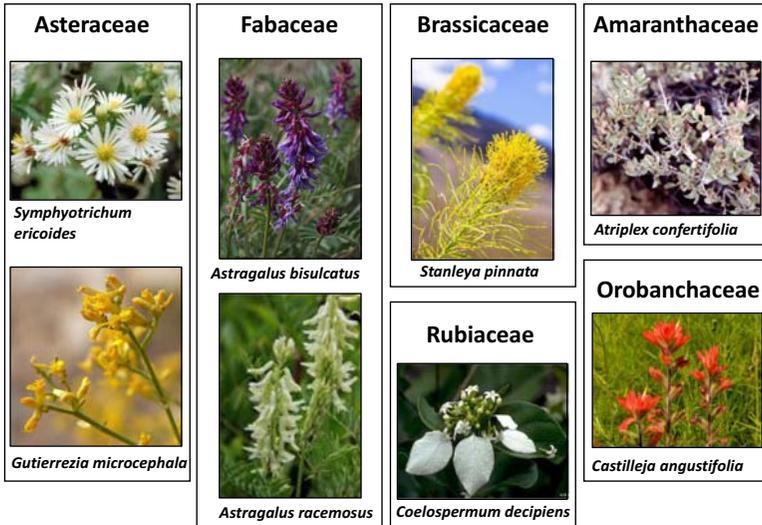
Furthermore, the Se concentration in their shoot tissues is often indicative of soil phytoavailable Se (Pilon-Smits, 2019).

### 8.2.3 Se-hyperaccumulating plant species

Se-hyperaccumulating species are rare and include plants with an extremely high ability to accumulate and tolerate Se (Pilon-Smits, 2019; White, 2016). They can contain more than 1 mg Se g<sup>-1</sup> DW in their shoots while growing in their native environment, which is generally restricted to seleniferous soils. The bioconcentration factor (BF) for these species is generally very high (e.g., over 800:1 in *Astragalus bisulcatus*), and it may perform better than the absolute concentration threshold in discriminating hyperaccumulators from non-accumulators (Statwick *et al.*, 2016). The BF, as well as the translocation factor (TF), often negatively correlates with the plant biomass in non-hyperaccumulators because of Se toxicity. Conversely, in hyperaccumulators, despite the apparent costs required to actively concentrate high Se in tissues through energy-dependent mechanisms, Se seems to stimulate plant growth (Statwick *et al.*, 2016).

The discovery of Se-hyperaccumulators was made by Orville and coworkers in the 1930s in the western United States (Rosenfeld & Beath, 1964), and Se-hyperaccumulators that populate natural seleniferous soils in these areas accumulate up to 15 mg Se g<sup>-1</sup> DW (Pilon-Smits, 2019). Since then, Se hyperaccumulation has been recorded in about 45 taxa (Cappa & Pilon-Smits, 2014; Pilon-Smits, 2019; White, 2016). Figure 8.1 depicts representative Se hyperaccumulator plants. The genus *Astragalus* of the Fabaceae family comprises 25 Se hyperaccumulator species, while the Asteraceae genera *Xylorhiza* and *Symphyotrichum* contain 3 Se hyperaccumulator species each (Table 8.1). In other cases, there are only one or two species per genus, such as *Stanleya pinnata* and *Stanleya bipinnata* from the genus *Stanleya*, *Cardamine violifolia* from the genus *Cardamine* of the Brassicaceae family, and *Neptunia amplexicaulis* from the genus *Neptunia* of the Fabaceae family (El Mehdawi and Pilon-Smits, 2012; Harvey *et al.*, 2020; Rosenfeld & Beath, 1964; White, 2016). Except for *Cardamine violifolia*, which is native to seleniferous soils in the Yutangba region in China, and *Neptunia amplexicaulis*, which grows in seleniferous soils in Queensland (Australia), Se-hyperaccumulators are commonly native to seleniferous soils of the Western USA (Both *et al.*, 2020; Harvey *et al.*, 2020; Pilon-Smits, 2017).

Species of the genera *Astragalus* and *Stanleya* are likely the strongest Se-hyperaccumulators, being able to accumulate up to 10 mg Se g<sup>-1</sup> when growing in soils with 2–10 µg Se g<sup>-1</sup> (Schiavon & Pilon-Smits, 2017a). However, it must be noted that within an Se-hyperaccumulating species there are populations that largely differ in their capacity to accumulate Se, as well as individuals within the same population (Pilon-Smits, 2017). Such differences are mainly due to genetic variability, local Se availability, and perhaps associated rhizosphere and endophytic microorganisms (Pilon-Smits, 2017).



**Figure 8.1** Representative Se-hyperaccumulators within different families. Photos available at: <https://naturalcommunities.net/products/symphyotrichum-ericoides-heath-aster>; [http://www.easterncoloradowildflowers.com/Astragalus\\_bisulcatus.htm](http://www.easterncoloradowildflowers.com/Astragalus_bisulcatus.htm); <https://www.shutterstock.com/it/search/stanleya+pinnata>; [https://en.wikipedia.org/wiki/Atriplex\\_confertifolia](https://en.wikipedia.org/wiki/Atriplex_confertifolia); [https://en.wikipedia.org/wiki/Gutierrezia\\_microcephala](https://en.wikipedia.org/wiki/Gutierrezia_microcephala); <https://www.prairiemoon.com/astragalus-racemosus-creamy-milk-vetch-prairie-moon-nursery.html>; [http://www.canbr.gov.au/cpbr/cd-keys/RFK7/key/VFK7/Media/Html/entities/Coelospermum\\_decipiens.htm](http://www.canbr.gov.au/cpbr/cd-keys/RFK7/key/VFK7/Media/Html/entities/Coelospermum_decipiens.htm); <https://www.fs.fed.us/wildflowers/plant-of-the-week/Castilleja-coccinea.shtml> (photo by Christopher David Benda).

### 8.2.4 Se uptake in Se-hyperaccumulators

Se-hyperaccumulators possess a high expression of specific transporters mediating Se uptake and possibly delivering to the shoot, which might justify their extremely high capacity to accumulate Se. Studies so far indicate that these plants can discriminate between Se and its analog S, while non-hyperaccumulators cannot. This assumption is based on the observation that tissue Se/S ratios displayed by these plants are higher compared to non-hyperaccumulators (Schiavon *et al.*, 2015). The mechanism of this hypothesized preference for Se over S by Se-hyperaccumulators has not been clarified yet, but the existence of a sulfate transporter with a higher affinity for Se over S has been recently proposed in *S. pinnata*. This Se-hyperaccumulator has elevated and constitutive expression of the gene encoding in the root for high affinity sulfate transporter *Sultr1;2*. This transporter in the non-hyperaccumulators usually plays a major role in sulfur primary uptake and is regulated by both sulfate availability and the S status of the plant, typically down-regulated by high sulfate concentration in the external

**Table 8.1** Species, distribution and maximum Se shoot concentration of several Se hyperaccumulators as retrieved by White (2016).

Species	Plant Distribution	Se Concentration (mg Se kg <sup>-1</sup> DW)
Asteraceae (Asterales)		
<i>Dieteria canescens</i> (Pursh) Nutt.	Midwest USA	1600
<i>Grindelia squarrosa</i> (Pursh) Dunal	Lower Brule Reservation, SD, USA	930
<i>Gutierrezia microcephala</i> (DC.) A.Gray	Thompson, UT, USA	1287
<i>Oonopsis foliosa</i> Greene	Lascar, CO, USA	3630
<i>Oonopsis wardii</i> (A.Gray) Greene	Albany County, WY, USA	9120
<i>Symphotrichum ascendens</i> (Lindl.) G.L.Nesom	Soda Springs, ID, USA	4455
<i>Symphotrichum ericoides</i> (L.) G.L.Nesom	Pine Ridge, Fort Collins, CO, USA	1378
<i>Symphotrichum lateriflorum</i> (L.) Á. Löve & D.Löve	SD, USA	1800
<i>Xylorhiza glabriuscula</i> Nutt.	Huerfano County, CO, USA	1750
<i>Xylorhiza parryi</i> Greene	Albany County, WY, USA	5390
<i>Xylorhiza venusta</i> (M.E.Jones) A. Heller	Midwest USA	3486
Fabaceae		
<i>Acacia cana</i> Maiden	NW Queensland, Australia	1121
<i>Astragalus albulus</i> Wooton & Standl.	La Ventana, NM, USA	530
<i>Astragalus beckwithii</i> var. <i>purpureus</i> M.E.Jones	Cameron, AZ, USA	3135
<i>Astragalus bisulcatus</i> (Hook.) A.Gray	Pine Ridge, Fort Collins, CO, USA	13,685
<i>Astragalus bisulcatus</i> var. <i>haydenianus</i> (A. Grey) Barneby	Cuba, NM, USA	2377
<i>Astragalus canadensis</i> L.	Las Vegas, NE, USA	1110
<i>Astragalus crotalariae</i> A.Gray	Truckhaven, CA, USA	2175
<i>Astragalus eastwoodiae</i> M.E.Jones	Utah, USA	1664
<i>Astragalus flavus</i> Torr. & A.Gray	Aztec, NM, USA	1361
<i>Astragalus flavus</i> var. <i>argillosus</i> (M.E.Jones) Barneby	Greenriver, UT, USA	631
<i>Astragalus flavus</i> var. <i>candicans</i> A.Gray	Thompson, UT, USA	1322
<i>Astragalus grayi</i> S.Watson	Carbon County, WY, USA	4450
<i>Astragalus osterhoutii</i> M.E.Jones	Kremmling, CO, USA	2678
<i>Astragalus pattersonii</i> A.Gray	Thompson, UT, USA	8512

(Continued)

**Table 8.1** Species, distribution and maximum Se shoot concentration of several Se hyperaccumulators as retrieved by [White \(2016\)](#) (*Continued*).

Species	Plant Distribution	Se Concentration (mg Se kg <sup>-1</sup> DW)
<i>Astragalus pectinatus</i> (Hook.) G.Don	Teton County, MT, USA	5170
<i>Astragalus praelongus</i> E.Sheld.	Leupp, AZ, USA	4835
<i>Astragalus praelongus</i> var. <i>ellisiae</i> (Rydb.) B.L.Turner	Valmont, NM, USA	656
<i>Astragalus preussii</i> A.Gray	Thompson, UT, USA	4188
<i>Astragalus racemosus</i> Pursh.	WY, USA	14,920
<i>Astragalus rafaensis</i> M.E.Jones	Jensen, TX, USA	716
<i>Astragalus sabulosus</i> M.E.Jones	Thompson, UT, USA	2210
<i>Astragalus toanus</i> M.E.Jones	ID, USA	990
<i>Neptunia amplexicaulis</i> Domin	Richmond, Queensland, Australia	4334
Brassicaceae (Brassicales)		
<i>Cardamine hupingshanensis</i>	Yutangba, Enshi, China	1965
<i>Cardamine violifolia</i>	Yutangba, China	2700
<i>Stanleya bipinnata</i> Greene	Laramie, WY, USA	2490
<i>Stanleya pinnata</i> (Pursh) Britton	Pine Ridge, Fort Collins, CO, USA	>4000
<i>Stanleya pinnata</i> var. <i>integrifolia</i> (E. James) Rollins	Vernal, UT, USA	977
Amaranthaceae (Caryophyllales)		
<i>Atriplex confertifolia</i> (Torr. & Frém.) S. Watson	Thompson, UT, USA	1734
<i>Atriplex nutallii</i> S.Watson	WY, USA	930
Rubiaceae (Gentianales)		
<i>Coelospermum decipiens</i> Baill.	Cape York Peninsula, Queensland, Australia	1141
Orobanchaceae (Lamiales)		
<i>Castilleja angustifolia</i> var. <i>dubia</i>	Lysite, WY, USA	3460

Se hyperaccumulators whose Se shoot concentration and distribution are unknown are not reported.

medium and in the plant ([El Kassis et al., 2007](#)). *Sultr1;2* from *S. pinnata* (*SpSultr1;2*) is not subjected to such a feedback inhibition by high sulfate concentrations ([El Mehdawi et al., 2018](#)). Furthermore, increased expression of *Sultr1;2* is associated with high Se uptake rates in this hyperaccumulator. Consistent with already described metal hyperaccumulation mechanisms ([Craciun](#)

*et al.*, 2012; Hanikenne *et al.*, 2008; Lochlainn *et al.*, 2011), the overexpression of *Sultr1;2* may be attributed in part to gene duplication events, which would account for the enhanced Se transport capacity and likely allowed at least one of the gene copies to evolve toward greater specificity for Se over S. Also, both the constitutively high expression and concomitant unresponsiveness of *SpSultr1;2* to S might have been the result of either mutation in cis-regulatory sequences of the transporter or changes in its coding sequence that may have altered the transporter affinity and specificity for the substrate (El Mehdawi *et al.*, 2018).

In addition to selenate, plants can take up organic Se species very efficiently. In a transcriptome study, the gene *LHT1* was substantially more expressed in *S. pinnata* than in the non-hyperaccumulator *Stanleya elata* (Wang *et al.*, 2018). *LHT1* encodes a high affinity transporter for cellular amino acid uptake from the soil into the mesophyll cells (Hirner *et al.*, 2006). Owing to their broad substrate specificity, *LHT1* proteins may mediate the uptake of Se amino acids, either methylated or not, which can be released to the soil once the plant residues from Se-hyperaccumulators are decomposed by soil microorganisms. Se-hyperaccumulators are generally rich in organic Se and their deposition may affect soil Se speciation (Pilon-Smits, 2019). Therefore, the upregulation of *LHT1* in these species might represent a strategy to get more highly available organic Se in the surrounding environment.

Once inside roots cells, inorganic Se can be delivered to the plastids to access the S-assimilation pathway, or loaded into the xylem and be transferred to the shoot for assimilation into the chloroplasts of mesophyll cells (Sors *et al.*, 2005). Organic Se produced in the roots or accumulated after uptake can be also conveyed to the shoot through the xylem flow. *S. pinnata* plants show high expression of the low-affinity sulfate transporter *Sultr2;1*, which may be, at least in part, responsible for the enhanced accumulation of Se in the shoot (El Mehdawi *et al.*, 2018).

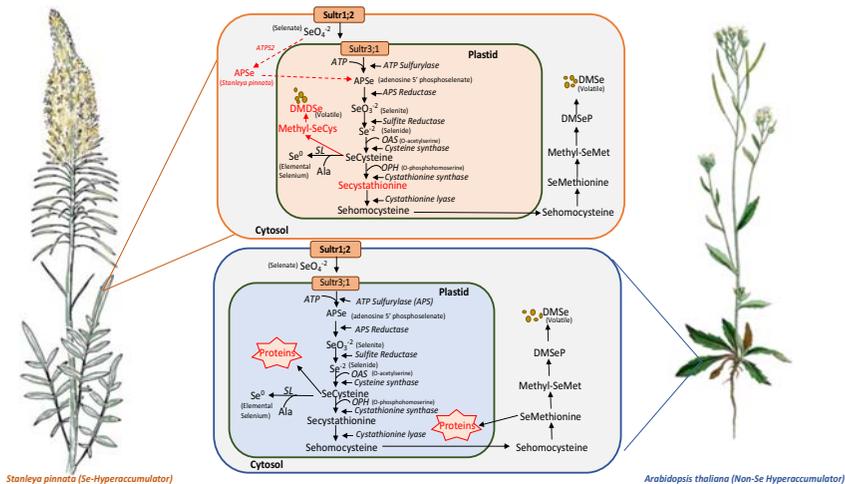
## 8.3 METABOLIC PATHWAYS SUPPORTING Se HYPERACCUMULATION

### 8.3.1 Se metabolism in non-hyperaccumulating plants

Selenium (Se) is not considered a nutrient for plants, but it can be beneficial to them in lower concentrations, and in contrast, it is toxic to most species at higher tissue levels. This antagonistic characteristic is puzzling and fascinating at the same time and establishes Se as a unique element when its interaction with plants is considered. Non-Se-hyperaccumulator plants take up and translocate Se inadvertently using sulfur transporters. The inorganic Se is then nonspecifically assimilated and reduced via sulfur metabolism to the amino acids SeCys (selenocysteine) and SeMet (selenomethionine), resulting in protein malfunction and systemic stress.

### 8.3.2 Organo-Se synthesis in hyperaccumulating plants

This scenario is, however, more complex for the Se-hyperaccumulator species. These plants evolved to cope with the high concentrations of Se in their tissues, therefore, they can accumulate thousands of mg Se kg<sup>-1</sup> DW without showing—any symptoms of stress. The main mechanism used by these plants to avoid the misincorporation of Se into proteins is elegant and efficient: the accumulation of organic forms of Se. This is accomplished by some different processes including methylation of the amino acid SeCys, and exclusion of Se by volatilization. The main forms of Se found in these plants are selenocystathionine and methylselenocysteine (MeSeCys), the latter is formed by a unique isoform of the enzyme SeCys methyltransferase (SMT) found in Se-hyperaccumulators (Freeman *et al.*, 2010). The methylated form of cysteine cannot be incorporated into proteins. Thus, it can be accumulated at high levels without inducing any stress symptoms (Figure 8.2). This enzyme is constitutively expressed in Se-hyperaccumulators, and previous studies showed that SMT from Se-hyperaccumulator species *Astragalus bisulcatus* can induce the accumulation of the organic and less toxic MeSeCys when expressed in the non-Se-hyperaccumulator plants *Arabidopsis thaliana* and *Brassica juncea*, resulting in tolerance to Se, confirming its important role in Se-hyperaccumulator species (LeDuc *et al.*, 2004).



**Figure 8.2** Selenium assimilation in hyperaccumulators and non Se-hyperaccumulators. Biochemical routes specific to hyperaccumulators are highlighted in red. ATPS2 = ATP sulfurylase isoform 2, SL = selenocysteine lyase, Ala = alanine, DMSe = dimethyl selenide, DMDS = dimethyl diselenide. Adapted from Schiavon and Pilon-Smits (2017a).

Another important and unique metabolic pathway found in the Se-hyperaccumulator plants is the ability to volatilize Se from the leaves. This process starts with the oxidation of the MeSeCys, forming methylselenocysteineselenideoxide (MeSeCysSeO) (Chin & Lindsay, 1994). MeSeCysSeO is then converted to methaneselenol by the enzyme cysteine sulfoxide lyase, which is then converted to dimethyldiselenide (DMDSe), a volatile form of selenium that can be excluded from the leaves (Valdez Barillas *et al.*, 2011). This process can protect the plant from the possible Se toxicity, but it is also hypothesized to help the plant to avoid herbivores through deterrence (Schiavon & Pilon-Smits, 2017a).

Se is stored in vacuoles of epidermal leaf cells, and it can be strategically stored in high concentrations in cells at the edge of the leaf blade in *S. pinnata*, possibly for herbivory protection (Freeman *et al.*, 2010). There is a seasonal variation in selenium accumulation in the *S. pinnata* leaves, where higher Se concentrations can be found in early spring and lower concentrations in the fall, while sulfur levels spike in midsummer (El Mehdawi & Pilon-Smits, 2012).

### 8.3.3 Enzymology of organo-Se formation

Most of the enzymes in the sulfur assimilation pathways are constitutively overexpressed in the Se-hyperaccumulator species when compared to non-Se-hyperaccumulators. The first enzyme in the selenate/sulfate assimilation is the enzyme adenosine triphosphate (ATP)-sulfurylase (ATPS), responsible for forming adenosine 5'phosphoselenate (APSe) from ATP and selenate/sulfate. This step is important and considered to be rate-limiting in the reduction of inorganic selenium to the less toxic organic forms (Pilon-Smits *et al.*, 1999). The ATPS enzyme represents a key step in the reduction process of selenate to selenite, the overexpression of this enzyme in *B. juncea* resulted in enhanced accumulation of organic Se, thus enhancing tolerance and accumulation of Se (Pilon-Smits *et al.*, 1999).

ATP-sulfurylase can be found in both the cytosol and the plastids in non-Se-hyperaccumulators (Pilon-Smits *et al.*, 1999). Four different isoforms are known in *A. thaliana*: the isoforms ATPS1-3-4 are found in plastids and the isoform ATPS2 has dual localization, cytosolic and plastidial. There are also some qualitative differences between the Se-hyperaccumulator *S. pinnata* and the non-Se-hyperaccumulators *S. elata* and *A. thaliana*. The Se-hyperaccumulator ATP sulfurylase isoform 2 (ATPS2) enzyme transcription is higher in roots, and it has a unique cytosolic localization (Figure 8.2), due to a stop codon in the DNA sequence coding for the protein, resulting in an interrupted chloroplast transit peptide in the amino acid sequence (Jiang *et al.*, 2018). This would indicate the selenium could be reduced in the root cells, and subsequently transported to the shoot in its organic forms in *S. pinnata*.

In the past 20 years, several different genes from the sulfur assimilation pathway have been manipulated to study their effect on plant Se tolerance and accumulation. The overexpression of the enzyme cystathionine synthase, responsible for the formation of Se cystathionine from SeCys (Figure 8.2), in *Brassica juncea* resulted in three-fold high Se volatilization from either selenate or selenite, indicating expression of this enzyme as a limiting factor for Se volatilization in plants (Van Huysen *et al.*, 2003). As stated before (see 8.3.2), the conversion of inorganic Se to its less toxic organic forms is the main step towards Se tolerance in Se-hyperaccumulator plants, and the enzyme SMT plays a crucial role in this process. LeDuc *et al.* (2004) demonstrated that overexpressing the SMT from the hyperaccumulator *A. bisulcatus* in the non-Se-hyperaccumulators *A. thaliana* and *B. juncea*, enhanced the Se volatilization, tolerance, and the accumulation of MeSeCys. The effect was more pronounced when the transgenic plants were supplied with selenite, which indicates the reduction of selenate to selenide is a limiting step in the assimilation pathway.

Another enzyme that was extensively studied is the selenocysteine lyase (SL), which breaks down SeCys into elemental Se and alanine (this process is widely used by non-Se-hyperaccumulators to detoxify the excess of Se). Some studies showed the overexpression of SL can reduce the incorporation of Se into proteins, enhance volatilization and overall tolerance in *A. thaliana*, and improve Se accumulation by two-fold in *B. juncea*, a promising trait for Se phytoremediation and fortification in crop fields (Bañuelos *et al.*, 2002, 2015).

The Se-hyperaccumulator plants also show tissue-specific patterns of Se accumulation. The Se levels are usually high in young leaves and reproductive tissues like the flower, silique, seeds, pollen, and ovules due to its remobilization from aging leaves (Cappa & Pilon-Smits, 2014).

## 8.4 EVOLUTION OF THE Se HYPERACCUMULATION TRAIT

### 8.4.1 Main driving-factors

The Se hyperaccumulation is a derived trait that possibly evolved independently in 45 different taxa, in 14 genera from 6 dicot plant families, including the Brassicaceae, Fabaceae, and Asteraceae families (White, 2016). Because the occurrence of Se-hyperaccumulators correlates with the Se content in the soil, usually in seleniferous areas, it can be hypothesized that the Se bioavailability, concentration, and distribution in the soil were possibly the most important driving factors for the evolution of this trait. However, these might not be the only aspects to consider when analyzing the evolution of Se hyperaccumulation, because just a small number of plant species found in seleniferous areas can tolerate extreme concentrations of Se. Therefore, the ecological benefits from the high selenium concentration, the upregulation of genes related to the antioxidant

activity and hormonal stress resistance were possibly strong components, in combination with seleniferous soils, driving the evolution of this trait (Schiavon & Pilon-Smits, 2017a).

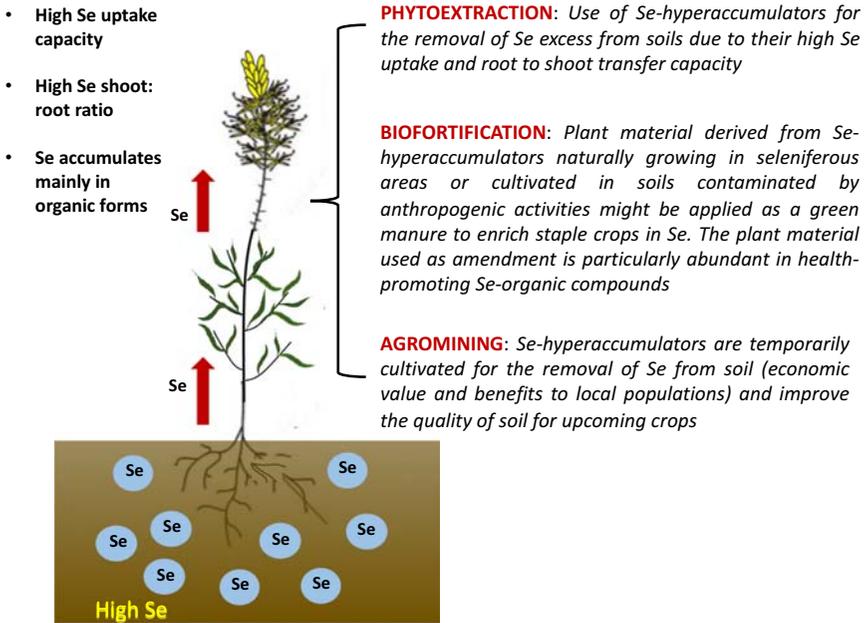
### 8.4.2 Metabolic defense mechanisms

The Se-hyperaccumulator species *S. pinnata* demonstrates a higher expression of genes involved in the biosynthesis of different hormones related to biotic and abiotic stress resistance, including jasmonic acid, ethylene, and salicylic acid, as well as an upregulation of different antioxidant enzymes, when compared to the non-Se-hyperaccumulator *S. elata* (Wang *et al.*, 2018). The cellular antioxidant defense mechanisms correspond to the enzymatic and non-enzymatic responses to directly quench the reactive oxygen species (ROS), such as the superoxide radical ( $O_2^-$ ) or hydrogen peroxide ( $H_2O_2$ ), thus neutralizing the deleterious effects of the different reactive processes. In other words, Se-hyperaccumulator plants are more prepared to deal with different stresses and are selected in evolution to tolerate and accumulate Se. It was found, for example, that genes involved in the synthesis of glutathione were upregulated in the Se-hyperaccumulator *S. pinnata* (Wang *et al.*, 2018). Glutathione is a tripeptide formed from the amino acids glutamate, cysteine and glycine, and is a major metabolite in the defense system against ROS and its degenerative effects during the antioxidant stress, which could be caused by high concentrations of Se.

### 8.4.3 Plant ecology

The ecological aspects of hyperaccumulation also played an important role in the evolution of the trait. It is known that the extreme Se concentrations found in the hyperaccumulator species *A. bisulcatus* and *S. pinnata* can promote protection against different herbivores due to deterrence (Quinn *et al.*, 2010), and pathogenic fungi in *B. Juncea* (Hanson *et al.*, 2003). There is evidence that hyperaccumulator plants can also benefit from the higher Se concentration when competing with neighbor plants, by increasing the Se concentration in the soil up to 10 times, due to litter deposition and exudation, which affects the germination and growth of Se-sensitive plants (El Mehdawi *et al.*, 2012).

Endophytic bacterial communities harbored by Se hyperaccumulators might also have played a role in the evolution of the Se hyperaccumulation and hypertolerance traits in these species and are different in composition compared to endophytic bacterial communities harbored by non-Se-accumulators (Cochran *et al.*, 2018). In a recent study, endophytic bacteria highly resistant to selenate and selenite (up to 200 mM) and able to reduce selenite to elemental Se, as well as to reduce nitrite and produce siderophores, have been isolated and identified in *S. pinnata* and *A. bisulcatus* plants growing in their natural environments (Sura de-Jong *et al.*, 2015). These isolates may increase the tolerance of plants to Se by reducing its potential toxicity in tissues, and provide them with additional



**Figure 8.3** Potential application of Se-hyperaccumulators (Schiavon & Pilon-Smits, 2017b).

benefits. Indeed, some of the isolates showed plant growth-promoting properties when inoculated in non-hyperaccumulators. In turn, organic forms of Se released by hyperaccumulators in their habitats may alter microbial species composition, favoring those taxa that utilize the essential nutrient Se more efficiently.

## 8.5 POTENTIAL USES OF Se-HYPERACCUMULATORS IN PHYTOTECHNOLOGIES

Se-hyperaccumulators find potential practical application in different types of phytotechnologies, such as (i) phytoextraction of Se from mining-wastes and reclamation of Se-polluted soils, (ii) biofortification of staple crops to combat Se deficiency in vulnerable populations living in low Se areas, and (iii) agromining (Figure 8.3) (Schiavon & Pilon-Smits, 2017b).

### 8.5.1 Phytoremediation

Selenium, similar to other metalloids (e.g., arsenic) and metals (e.g., thallium, cobalt, cadmium, and rhenium), is a by-product occurring in mining wastes that may be at concentrations suitable for economic phytoextraction (phytomining). Se phytoextraction could be carried out in those areas where wastes from Se-rich

coal represent a potential threat to the environment (Reeves *et al.*, 2018). Such an approach has been adopted in the USA, Canada, the UK, and Australia (Remigio *et al.*, 2020), and has also been applied to soils that are contaminated with Se due to extensive use of Se-rich irrigation waters. Se-hyperaccumulators might be ideally used in Se phytoextraction of Se contaminated substrates in areas where they are naturally widespread. Thus, *A. bisulcatus* or *S. pinnata* could be used in the USA, whereas *C. violifolia* and *N. amplexicaulis* could be used in China and Australia, respectively.

The great capacity of Se-hyperaccumulators to concentrate Se at extremely high concentrations in their shoot and convert it into relatively less-toxic, human health beneficial organic forms, make them useful for phytoextraction, as harvesting could be restricted only to the above-ground parts of the plant. Furthermore, the Se-hyperaccumulator *S. pinnata* has been shown to volatilize Se at high rates when used for the remediation of Se-rich irrigation wastewater under high sulfate conditions (Parker *et al.*, 2003). Because of this efficient Se metabolism, *S. pinnata* has been proposed as a promising species to use for Se phytoremediation. Similarly, the hyperaccumulator *A. bisulcatus* substantially enhanced the emission rate of Se from soil (Pletsch, 2003). Freeman and Bañuelos (2011) found that hyperaccumulation and volatilization of Se by *S. pinnata* genotypes and their associated microbes could remove approximately 30% of the total soil Se in 0–30 cm Se-laden agricultural drainage sediment containing  $9.0 (\pm 3.8) \mu\text{g Se g}^{-1} \text{DW}$ . However, despite these promising results and the advantages offered by Se-hyperaccumulators, so far only a few large field-scale studies have been conducted using these plants, mainly because of their non-agronomic status, coupled with their low biomass yield and slow growth (Zambrano *et al.*, 2018). Also, significant differences in Se accumulation might exist between different ecotypes of Se hyperaccumulators, as evidenced for *S. pinnata* by Feist and Parker (2001). Rather, Se accumulators (e.g., *Brassica juncea* L. Czern) producing high biomass yields and of moderate capacity to take up and translocate Se to the above-ground tissues have been widely employed (Bañuelos *et al.*, 2002; Dhillon & Bañuelos, 2017).

### 8.5.2 Biofortification

Se-hyperaccumulators growing in natural seleniferous areas or used for the reclamation of Se-contaminated soils can be a source of Se-laden plant material to be employed as green manure to enhance the content of Se in agricultural soils and food crops (Bañuelos *et al.*, 2015, 2016; Schiavon & Pilon-Smits, 2017b; Stonehouse *et al.*, 2020). Bañuelos *et al.* (2015, 2016) showed that Se-enriched plant material from *S. pinnata* plants, initially grown to remove Se by phytoextraction from Se-laden agricultural drainage sediment, is valuable as a soil amendment for enriching broccoli and carrots with healthful organic-Se compounds, especially SeMet.

The plant material derived from Se-hyperaccumulators can otherwise be recycled as forage for livestock (Bañuelos *et al.*, 2009). In this way, Se phytoremediation and Se biofortification technologies can be proficiently joined. However, before being launched into the food chain, Se-laden material from Se-hyperaccumulators must be subjected to rigorous controls to verify the absence of potential contaminants (e.g., heavy metals and metalloids) that might be toxic to direct consumers (livestock), crops, and food crop consumers (humans).

Se-hyperaccumulators are particularly rich in Se organic forms (e.g., SeMet and MetSeCys). Therefore, crops to be amended with green manure obtained from Se-hyperaccumulators could be selected via breeding or otherwise engineered for elevated uptake rates and accumulation of organic Se in the edible produce. Food crops enriched with organic Se compounds might possess higher nutritional values compared to crops that principally accumulate inorganic Se, as organic Se compounds are more readily available for essential Se metabolism in humans and animals (Davis, 2012). Alternatively, Se-hyperaccumulators could be used in co-cropping or intercropping (Schiavon & Pilon-Smits, 2017b). In this case, their residues will deposit on the soil at the end of the growing season, and their decomposition by Se-resistant soil microorganisms will lead to the release of Se organic compounds that might be easily acquired by the neighboring or upcoming crops. The microbiome of Se-hyperaccumulators has been recently found to be different from that of non-hyperaccumulators, with a higher average relative abundance of *Pedobacter* and *Deviosa* (Cochran *et al.*, 2018).

### 8.5.3 Agromining

Compared to other Se phytotechnologies, Se agromining as an alternative type of agriculture conducted on low productive agricultural (ultramafic) lands is poorly widespread. Agromining is a variation of phytomining, which foresees the cultivation of hyperaccumulators and aims to harvest their biomass to retrieve target metal/loids. The recovery of metal/loids (bio-ores) involves the drying, ashing, and managing of the plant biomass. This is an important process in view of the fact that ore grades are progressively declining, resulting in increased waste products from the mining industry. Agromining is economically viable only for certain metal/loids, including Se, nickel (Ni), and tellurium (Te), as it depends on the element market price, annual yield per unit area, and the existence of regions enriched in the target element. Till now, prices per metric ton are high for Ni (US\$14,000), Se (US\$44,000), and Te (US\$65,000) (USGS, 2020).

The idea of growing hyperaccumulators on infertile soils raises from the consideration that most of them are highly nutrient-efficient, and thus have low fertilization requirements. Agromining might provide better economic incomes to local populations and should be intended as temporary farming for metal/loids and not as a steady replacement of food crops. Also, after hyperaccumulators are

harvested for metal/loid recovery, the soil fertility is supposed to have improved enough by their cultivation to support the growth of upcoming crops (van der Ent *et al.*, 2015). Despite the benefits of using hyperaccumulators for agromining, the adoption of Se-hyperaccumulators for economic extraction of elemental Se is still a very limited practice.

## 8.6 CONCLUSION

Se-hyperaccumulators are intriguing species characterized by an extraordinary capacity to accumulate Se in all their tissues and a higher transfer capacity of Se from the roots to their shoot. Research performed so far has highlighted the potential evolutionary drivers and selective pressures responsible for the development of the hyperaccumulation trait, and the ecological benefits associated with high Se concentrations in tissues. Pieces of evidence are suggesting that Se-hyperaccumulators possess specific mechanisms for Se uptake and root to shoot translocation, while the specific metabolic routes through which they prevent Se amino acid misincorporation in proteins have been widely described. Ongoing studies aimed to overexpress genes from hyperaccumulators into non-hyperaccumulator model species may help to elucidate the process of hypertolerance and hyperaccumulation in plants. These genes could be further transferred into plant species suitable for phytoremediation, biofortification, and agromining. Indeed, although Se-hyperaccumulators may be employed in these technologies, their use in field application is unfortunately still limited primarily because of their limited biomass production, which is generally smaller compared to that produced by non-Se accumulators.

## REFERENCES

- Bañuelos G. S., Lin Z. Q., Wu L. and Terry N. (2002). Phytoremediation of selenium-contaminated soils and waters: fundamentals and future prospects. *Reviews on Environmental Health*, **17**(4), 291–306.
- Bañuelos G. S., Robinson J. and Da Roche J. (2009). Developing selenium-enriched animal feed and biofuel from canola planted for managing Se-laden drainage waters in the Westside of Central California. *International Journal of Phytoremediation*, **12**, 243–254.
- Bañuelos G. S., Arroyo I., Pickering I. J., Yang S. I. and Freeman J. L. (2015). Selenium biofortification of broccoli and carrots grown in soil amended with Se-enriched hyperaccumulator *Stanleya pinnata*. *Food Chemistry*, **166**, 603–608.
- Bañuelos G. S., Arroyo I. S., Dangi S. R. and Zambrano M. C. (2016). Continued selenium biofortification of carrots and broccoli grown in soils once amended with Se-enriched *S. pinnata*. *Frontiers in Plant Science*, **23**, 7–1251.
- Both E. B., Stonehouse G. C., Lima L. W., Fakra S. C., Aguirre B., Wangeline A. L., Xiang J., Yin H., Jókai Z., Soós Á., Dernovics M. and Pilon-Smits E. A. H. (2020). Selenium tolerance, accumulation, localization and speciation in a *Cardamine*

- hyperaccumulator and a non-hyperaccumulator. *Science of the Total Environment*, **703**, 135041.
- Cappa J. J. and Pilon-Smits E. A. H. (2014). Evolutionary aspects of elemental hyperaccumulation. *Planta*, **239**, 267–275.
- Chauhan R., Awasthi S., Srivastava S., Dwivedi S., Pilon-Smits E. A. H., Dhankher O. P. and Rudra D. (2019). Understanding selenium metabolism in plants and its role as a beneficial element. *Critical Reviews in Environmental Science and Technology*, **49**, 1937–1958.
- Chin H. W. and Lindsay R. C. (1994). Mechanisms of formation of volatile sulfur compounds following the action of cysteine sulfoxide lyases. *Journal of Agricultural and Food Chemistry*, **42**, 1529–1536.
- Cochran A. T., Bauer J., Metcalf J. L., Lovecka P., Sura de Jong M., Warris S., Mooijman P. J. W., van der Meer I., Knight R. and Pilon-Smits E. A. H. (2018). Plant selenium hyperaccumulation affects rhizosphere: enhanced species richness and altered species composition. *Phytobiomes*, **2**, 82–91.
- Craciun A. R., Meyer C.-L., Chen J., Roosens N., De Groodt R., Hilson P. and Verbruggen N. (2012). Variation in HMA4 gene copy number and expression among *Noccaea caerulea* populations presenting different levels of Cd tolerance and accumulation. *Journal of Experimental Botany*, **63**, 4179–4189.
- Davis C. D. (2012). Selenium supplementation and cancer prevention. *Current Nutrition Reports*, **1**, 16–23.
- Dhillon K. S. and Bañuelos G. S. (2017). Overview and prospects of selenium phytoremediation approaches. In: *Selenium in Plants: Molecular, Physiological, Ecological and Evolutionary Aspects*, E. A. H. Pilon-Smits, L. H. E. Winkel and Z.-Q. Lin (eds.), Springer International Publishing, Switzerland, pp. 277–321.
- El Kassis E., Cathala N., Rouached H., Fourcroy P., Berthomieu P., Terry N. and Davidian J. C. (2007). Characterization of a selenate-resistant *Arabidopsis* mutant. Root growth as a potential target for selenate toxicity. *Plant Physiology*, **3**, 1231–1241.
- El Mehdawi A. F., Cappa J. J., Fakra S. C., Self J. and Pilon-Smits E. (2012). Interactions of selenium hyperaccumulators and nonaccumulators during cocultivation on seleniferous or nonseleniferous soil – the importance of having good neighbors. *The New Phytologist*, **194**(1), 264–277.
- El Mehdawi A. F. and Pilon-Smits E. A. H. (2012). Ecological aspects of plant selenium hyperaccumulation. *Plant Biology*, **14**, 1–10.
- El Mehdawi A. F., Jiang Y., Guignardi Z. S., Esmat A., Pilon M., Pilon-Smits E. A. H. and Schiavon M. (2018). Influence of sulfate supply on selenium uptake dynamics and expression of sulfate/selenate transporters in selenium hyperaccumulator and nonhyperaccumulator Brassicaceae. *New Phytologist*, **217**, 194–205.
- Feist L. J. and Parker D. R. (2001). Ecotypic variation in selenium accumulation among populations of *Stanleya pinnata*. *New Phytologist*, **149**, 61–69.
- Freeman J. L. and Bañuelos G. S. (2011). Selection of salt and boron tolerant selenium hyperaccumulator *Stanleya pinnata* genotypes and characterization of Se phytoremediation from agricultural drainage sediments. *Environmental Science & Technology*, **22**, 9703–9710.
- Freeman J. L., Tamaoki M., Stushnoff C., Quinn C. F., Cappa J. J., Devonshire J., Fakra S. C., Marcus M. A., McGrath S. P., Van Hoewyk D. and Pilon-Smits E. A. H. (2010).

- Molecular mechanisms of selenium tolerance and hyperaccumulation in *Stanleya pinnata*. *Plant Physiology*, **153**(4), 1630–1652.
- Hanikenne M., Talke I. N., Haydon M. J., Lanz C., Nolte A., Motte P., Kroymann J., Weigel D. and Kraemer U. (2008). Evolution of metal hyperaccumulation required cis-regulatory changes and triplication of HMA4. *Nature*, **453**, 391–395.
- Hanson B. R., Garifullina G. F., Lindblom S. D., Wangeline A., Ackley A., Kramer K., Norton A. P., Lawrence C. B. and Pilon-Smits E. A. H. (2003). Selenium accumulation protects *Brassica juncea* from invertebrate herbivory and fungal infection. *New Phytologist*, **159**, 461–469.
- Hartikainen H. (2005). Biogeochemistry of selenium and its impact on food chain quality and human health. *Journal of Trace Elements in Medicine and Biology*, **18**, 309–318.
- Harvey M. A., Erskine P. D., Harris H. H., Brown G. K., Pilon-Smits E. A. H., Casey L. W., Echevarria G. and van der Ent A. (2020). Distribution and chemical form of selenium in *Neptunia amplexicaulis* from Central Queensland, Australia. *Metallomics*, **12**, 514–527.
- Hirner A., Ladwig F., Stransky H., Okumoto S., Keinath M., Harms A., Frommer W. B. and Koch W. (2006). Arabidopsis LHT1 is a high-affinity transporter for cellular amino acid uptake in both root epidermis and leaf mesophyll. *Plant Cell*, **18**(8), 1931–1946.
- Jiang Y., Schiavon M., Lima L. W., Tripti Jones R. R., El Mehdawi A. F., Royer S., Zeng Z., Hu Y., Pilon-Smits E. A. H. and Pilon M. (2018). Comparison of ATP sulfurylase 2 from selenium hyperaccumulator *Stanleya pinnata* and non-accumulator *Stanleya elata* reveals differential intracellular localization and enzyme activity levels. *Biochimica et Biophysica Acta – General Subjects*, **1862**(11), 2363–2371.
- Kamran M., Parveen A., Ahmar S., Malik Z., Hussain S., Chattha M. S., Saleem M. H., Adil M., Heidari P. and Chen J. T. (2019). An Overview of hazardous impacts of soil salinity in crops, tolerance mechanisms, and amelioration through selenium supplementation. *International Journal of Molecular Sciences*, **21**(1), 148.
- Kieliszek M. (2019). Selenium-fascinating microelement, properties and sources in food. *Molecules*, **24**(7), 1298.
- LeDuc D. L., Tarun A. S., Montes-Bayon M., Meija J., Malit M. F., Wu C. P., AbdelSamie M., Chiang C. Y., Tagmount A., deSouza M., Neuhierl B., Böck A., Caruso J. and Terry N. (2004). Overexpression of selenocysteine methyltransferase in *Arabidopsis* and Indian mustard increases selenium tolerance and accumulation. *Plant Physiology*, **135**, 377–383.
- Lima L. W., Pilon-Smits E. A. H. and Schiavon M. (2018). Mechanisms of selenium hyperaccumulation in plants: a survey of molecular, biochemical and ecological cues. *Biochimica et Biophysica Acta – General Subjects*, **1862**(11), 2343–2353.
- Lochlainn S. Ó., Bowen H. C., Fray R. G., Hammond J. P., King G. J., White P. J., Graham N. S. and Broadley M. R. (2011). Tandem quadruplication of HMA4 in the zinc (Zn) and cadmium (Cd) hyperaccumulator *Noccaea caerulescens*. *PLoS One*, **6**, e17814.
- Natasha Shahid M., Niazi N. K., Khalid S., Murtaza B., Bibi I. and Rashid M. I. (2018). A critical review of selenium biogeochemical behavior in soil-plant system with an inference to human health. *Environmental Pollution*, **234**, 915–934.
- Oldfield J. E. (2002). Selenium world atlas: Updated Edition; Selenium-Tellurium Dev. Assoc.: Grimbergen, Belgium, pp. 1–59.

- Parker D., Feist L., Varvel T., Thomason D. and Zhang Y. (2003). Selenium phytoremediation potential of *Stanleya pinnata*. *Plant Soil*, **249**(1), 157–165.
- Pilon-Smits E. A. H. (2017). Mechanisms of plant selenium hyperaccumulation. In: Selenium in Plants: Molecular, Physiological, Ecological and Evolutionary Aspects, E. A. H. Pilon-Smits, L. H. E. Winkel and Z.-Q. Lin (eds.), Springer International Publishing, Switzerland, pp. 53–66.
- Pilon-Smits E. A. H. (2019). On the ecology of selenium accumulation in plants. *Plants (Basel)*, **30**, 197.
- Pilon-Smits E. A. H., Hwang S. B., Lytle C. M., Zhu Y. L., Tai J. C., Bravo R. C., Leustek T. and Terry N. (1999). Overexpression of ATP sulfurylase in *Brassica juncea* leads to increased selenate uptake, reduction and tolerance. *Plant Physiology*, **119**, 123–132.
- Pletsch M. (2003). Plants and the environment. In: Phytoremediation, B. Thomas (ed.), Encyclopedia of Applied Plant Sciences, Elsevier, pp. 781–786.
- Quinn C. F., Freeman J. L., Reynolds R. J. B., Cappa J. J., Fakra S. C., Marcus M. A., Lindblom S. D., Quinn E. K., Bennett L. E. and Pilon-Smits E. A. H. (2010). Selenium hyperaccumulation offers protection from cell disruptor herbivores. *Plant Physiology*, **153**, 1630–1652.
- Reeves R. D., van der Ent A. and Baker A. J. M. (2018). Global distribution and ecology of hyperaccumulator plants. In: Agromining: Farming for Metals, Mineral Resource Reviews, A. van der Ent, R. D. Reeves and A. J. M. Baker (eds.), Springer International Publishing AG, pp. 75–92.
- Remigio A., Chaney R. L., Baker A. J. M., Edraki M., Erskine P. D., Echevarria G. and van der Ent A. (2020). Phytoextraction of high value elements and contaminants from mining and mineral wastes: opportunities and limitations. *Plant Soil*, **449**, 11–37.
- Rosenfeld I. and Beath O. A. (1964). Selenium: Geobotany, Biochemistry, Toxicity, and Nutrition. Academic Press, New York.
- Schiavon M. and Pilon-Smits E. A. (2017a). The fascinating facets of plant selenium accumulation – biochemistry, physiology, evolution and ecology. *New Phytologist*, **213**(4), 1582–1596.
- Schiavon M. and Pilon-Smits E. A. H. (2017b). Selenium biofortification and phytoremediation phytotechnologies: a review. *Journal of Environmental Quality*, **46**, 10–19.
- Schiavon M., Pilon M., Malagoli M. and Pilon-Smits E. A. H. (2015). Exploring the importance of sulfate transporters and ATP sulphurylases for selenium hyperaccumulation—a comparison of *Stanleya pinnata* and *Brassica juncea* (Brassicaceae). *Frontiers in Plant Science*, **6**, 2.
- Shahid M., Niazi N. K., Khalid S., Murtaza B., Bibi I. and Rashid M. I. (2018) A critical review of selenium biogeochemical behavior in soil-plant system with an inference to human health. *Environmental Pollution*, **234**, 915–934.
- Sors T. G., Ellis D. R. and Salt D. E. (2005). Selenium uptake, translocation, assimilation and metabolic fate in plants. *Photosynthesis Research*, **86**(3), 373–389.
- Statwick J., Majestic B. J. and Sher A. A. (2016). Characterization and benefits of selenium uptake by an *Astragalus hyperaccumulator* and a non-accumulator. *Plant Soil*, **404**, 345–359.
- Stonehouse G. C., McCarron B. J., Guignardi Z. S., El Mehdawi A. F., Lima L. W., Fakra S. C. and Pilon-Smits E. A. H. (2020). Selenium Metabolism in Hemp (*Cannabis*

- sativa* L.). Potential for Phytoremediation and Biofortification. *Environmental Science & Technology*, **54**(7), 4221–4230.
- Sura-de Jong M., Reynolds R. J., Richterova K., Musilova L., Staicu L. C., Chochoolata I., Cappa J. J., Taghavi S., van der Lelie D., Frantik T., Dolinova I., Strejcek M., Cochran A. T., Lovecka P. and Pilon-Smits E. A. (2015). Selenium hyperaccumulators harbor a diverse endophytic bacterial community characterized by high selenium resistance and plant growth promoting properties. *Frontiers in Plant Science*, **6**, 113.
- USGS (2020). National Minerals Information Center. pubs.usgs.gov (accessed 8 October 2020).
- Valdez Barillas J. R., Quinn C. F. and Pilon-Smits E. A. (2011). Selenium accumulation in plants—phytotechnological applications and ecological implications. *International Journal of Phytoremediation*, **13**(Suppl 1), 166–178.
- van der Ent A., Baker A. J. M., Reeves R. D., Pollard A. J. and Schat H. (2015). Commentary: Toward a more physiologically and evolutionarily relevant definition of metal hyperaccumulation in plants. *Frontiers in Plant Science*, **6**, 554.
- Van Hoewyk D. (2013). A tale of two toxicities: malformed selenoproteins and oxidative stress both contribute to selenium stress in plants. *Annals of Botany*, **112**(6), 965–972.
- Van Huysen T., Abdel-Ghany S., Hale K. L., LeDuc D., Terry N. and Pilon-Smits E. A. H. (2003). Overexpression of cystathionine- $\gamma$ -synthase enhances selenium volatilisation in *Brassica juncea*. *Planta*, **218**, 71–78.
- Wang J., Cappa J. J., Harris J. P., Edger P. P., Zhou W., Pires J. C., Adair M., Unruh S. A., Simmons M. P., Schiavon M. and Pilon-Smits E. A. H. (2018). Transcriptome-wide comparison of selenium hyperaccumulator and nonaccumulator *Stanleya* species provides new insight into key processes mediating the hyperaccumulation syndrome. *Plant Biotechnology Journal*, **16**, 1582–1594.
- White P. J. (2016). Selenium accumulation by plants. *Annals of Botany*, **117**(2), 217–235.
- White P. J. (2018). Selenium metabolism in plants. *Biochimica et Biophysica Acta – General Subjects*, **1862**(11), 2333–2342.
- Winkel L. H., Vriens B., Jones G. D., Schneider L. S., Pilon-Smits E. and Bañuelos G. S. (2015). Selenium cycling across soil-plant-atmosphere interfaces: a critical review. *Nutrients*, **7**(6), 4199–4239.
- Zambrano M. C., Yuan L., Yin X. and Bañuelos G. (2018). Element case studies: selenium. In: *Agromining: Farming for Metals Extracting Unconventional Resources Using Plants*, A. van der Ent, G. Echevarria, A. J. M. Baker and J. L. Morel (eds.), Springer International Publishing, Cham, pp. 241–251.