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Selenium biofortification in crops and its cycling in the agroecosystem

DOCTORAL THESIS

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ACADEMIC DISSERTATION

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**Dedicated to my family:
My parents, my sibling, my spouse
Tariq and our lovely boy Madyar**

پیشکش بہ باوک و دایک، خوشک و براک انم، ہر وہ ما بو ماوسہ ری

ژیانم تاریق و کورہ نازیزہ کھمان مادیار گیان

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Mervi Seppänen, Helinä Hartikainen and Juha Kontturi contributed to the research plan. The greenhouse work was carried out by Juha Kontturi. Measurements and laboratory analyses of selenium and the gene expression analyses by RT-qPCR were carried out by Nashmin Ebrahimi and Juha Kontturi. Selenium speciation analysis was carried out in Spain by Isabel Lopez Heras, Yolanda Madrid and Camron Camara. Statistical data analysis was done by Nashmin Ebrahimi. Helinä Hartikainen, Mervi Seppänen and Nashmin Ebrahimi were responsible for the writing of the manuscript and incorporation of the input of other authors.

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ABBREVIATIONS

| | |
|----------------------------------|---|
| AAP | Amino acid permeases |
| AAS | Atomic absorbance spectrometry |
| APR | Adenosine phosphosulfuryl reductase |
| APS | ATP-sulfurylase |
| APSe | Adenosine phosphoselenate |
| AtSULTRs | Sulfate transporters in <i>Arabidopsis thaliana</i> |
| CBL | Cystathionine β -lyase |
| CGS | Cystathionine γ -synthase |
| DM | Dry matter |
| DMDSe | Dimethyl diselenide |
| DMSe | Dimethyl selenide |
| Exp | Experiment |
| FAO | Food and Agriculture Organization |
| GG-SeMet | γ -glutamyl-selenomethionine |
| GG-SeMSeCys | γ -glutamyl-selenomethylselenocysteine |
| GPX | Glutathione peroxidase |
| GSH | Reduced glutathione |
| HMT | Homocysteine methyl transferase |
| HPLC-ICP-MS | High performance liquid chromatography - inductively coupled plasma - mass spectroscopy |
| ICP-OES | Inductively coupled plasma atomic emission spectroscopy |
| LHT | Lysine-Histidine like transporters |
| MMT | Methionine methyltransferase |
| MTR | Methionine synthase |
| Na ₂ SeO ₄ | Sodium selenate |
| OAS | O-acetyl serine |
| OsNIP | Nodulin 26-like intrinsic proteins in <i>Oryza sativa</i> |
| OsPT | Phosphorus transporters in <i>Oryza sativa</i> |
| ProT | Protein transporters |

| | |
|--------------------------------|---|
| RCBD | Randomized complete block design |
| RDA | Recommended dietary allowance |
| ROS | Reactive oxygen species |
| SeCys | Selenocysteine |
| SeedTF | Seed translocation factor (ratio of Se content of seeds to that of shoots) |
| SeFR | Selenium fertilizer recovery (ratio of Se content of harvested parts to the amount of applied Se) |
| SeHCys | Selenohomocysteine |
| SeMet | Selenomethionine |
| SeMSeCys | Selenomethylselenocysteine |
| SeO ₃ ²⁻ | Selenite |
| SeO ₄ ²⁻ | Selenate |
| SEPs | Sequential extraction procedures |
| SeUPE | Selenium uptake efficiency |
| SiR | Sulfite reductase |
| SL | Selenocysteine lyase |
| SMT | Selenocysteine methyl transferase |
| TF | Translocation factor (ratio of Se content of shoots to that of roots) |
| TR | Thioredoxin reductase |
| WHC | Water holding capacity |

ABSTRACT

Selenium (Se) is an essential element in the nutrition of humans and animals due to its core function in important enzymes and as a component of some proteins. Plants take up and metabolize Se in the same pathway as sulfur (S) because these elements have similar chemical and physical properties. At low dosage, Se is a beneficial element for the growth and development of plants, especially under stress conditions. However, its necessity for a plant's life cycle has not been proved. Agronomic biofortification is a solution to increase daily nutrient intake in human through the application of mineral micronutrient fertilizers to increase their content in edible parts of crops. In agronomic biofortification via Se fertilizer, plants play an important role in providing the food chain with Se. As compared with inorganic Se, organic Se compounds in plants are less toxic even at higher concentration in human and animal tissues. Most plants accumulate selenomethionine (SeMet) and some Se-accumulators produce selenomethylselenocysteine (SeMSeCys) and its γ -glutamyl derivative. These nonprotein selenoamino acids have anticarcinogenic and antitumor properties in animals and humans. Therefore, the use of plants with high activity of homocysteine/selenocysteine methyl transferase (HMT/SMT) enzyme in biofortification to produce these organic Se compounds is desirable.

In Finland, soils are naturally low in bioavailable Se, so some fertilizers have been amended since 1984 with selenate to counteract the negative impact of low Se intake on the health of humans and animals. The uptake, remobilization and loading of Se into seeds are strongly dependent on various factors such as soil properties, Se species and their content in the soil as well as plant species and climatic conditions. Moreover, the recovery of fertilizer Se is low (5-35%) and most applied Se probably remains in soils. The bottlenecks in Se biofortification pathways are not fully understood, so further studies are needed to investigate these bottlenecks and the fate of applied Se in the agroecosystem.

The aims of the present study were to identify the bottlenecks in the Se biofortification pathway and to assess the potential of Se-enriched plant residues in soil as Se sources for plants. An additional aim was to investigate the assimilation pathways of Se and its speciation in a biofortified oilseed rape crop. Furthermore, other important crops (wheat and forage grasses) were studied to compare their efficiency (ratio of Se in harvested parts into its application) of Se fertilizer recovery and biofortification under field conditions.

Results from a greenhouse experiment indicated that oilseed rape plants accumulated Se ($18.7 \mu\text{g g}^{-1}$) in their leaves 6 h after soil application of selenate. As expected, 80% of the Se that accumulated in the leaves was in the form of selenate. At 14 days after treatment, the two most accumulated Se species in the leaves were selenate (64%) and SeMet (29%). Eventually, SeMet was the most accumulated Se species (54 - 96%) in the seeds and meal. Six hours after Se treatment only 4% of Se accumulated in the leaves was in the labile form of SeMSeCys. The homology of the amino acid sequences of the SMT enzyme in oilseed rape with *B. oleracea* and *Astragalus*, indicated its similarity with non-accumulator *B. oleracea* plants with dual

HMT/SMT activity and this probably resulted in the transience of SeMSeCys accumulation.

Further study on the application of various Se forms (selenate and Se-enriched plant residues) illustrated that Se-enriched plant residues in the form of leaf or straw were not as effective as selenate in increasing the Se concentration in oilseed rape plants. Slow decomposition of plant residues in the soil may result in insufficient Se accumulation in plants. However, selenate application ($7 \mu\text{g kg}^{-1}$) with soil autoclaving resulted in significantly higher Se concentration in the seeds and higher Se fertilizer recovery in plants as compared with those cultivated on non-autoclaved soil. It is speculated that heating and autoclaving released Se from adsorbed surfaces into the soil solution.

In field conditions, higher rainfall and lower mean temperature in 2012 than in 2011 contributed to a severe reduction of Se fertilizer recovery in wheat and oilseed rape plants. The higher water content of soil can cause the leaching of selenate or its conversion to reduced forms. In addition, mixed forage grasses (timothy and red clover) as leafy crops had higher Se fertilizer recovery (up to 64% in the first cut and 19% in the second cut) than wheat and oilseed rape crops. Thus, Se application to forage grasses is the most effective tool in a Se biofortification program due to their higher capacity to transfer Se into the food chain.

Monitoring translocation and remobilization of Se during different developmental stages showed that Se can be translocated to the reproductive parts from senescing leaves and stems, but its loading from silique walls into seeds is inefficient. Based on the data, two bottlenecks in the Se biofortification pathway were identified. The first is Se bioavailability in soil and the second is its limited loading into the seeds. Further molecular and physiological studies are needed to reveal the mechanisms behind these bottlenecks for improving biofortification strategies. The long-term analyses of inorganic and organic Se pools in soil showed that the application of 160 kg ha^{-1} NPK fertilizer enriched with selenate in the recommended rate in Finland does not result in Se accumulation in soil.

Results based on oilseed rape plants in a sub-boreal agroecosystem showed that under optimum climatic conditions less than 50% of applied inorganic Se was taken up by plants and more than half remained in the soil or was volatilized into the atmosphere. In addition, only a small portion (14%) was recovered in harvested parts and the rest of the Se taken up was returned into the soil as plant residues. It was found that Se-enriched plant residues were not efficient in Se recycling. Generally, it can be concluded that the use of inorganic Se at the recommended rate and in forage grasses in Finnish agroecosystem is the most effective way to improve the Se intake by humans (indirectly) and animals (directly).

1. INTRODUCTION

Selenium (Se) is a widespread trace element discovered by Swedish chemist Jöns Jacob Berzelius in 1817 (Mehdi et al. 2013). This metalloid is widely used for many industrial purposes and products such as pharmaceuticals, paint pigments, glasses, photovoltaic cells, ceramics and semiconductors because of its ability to convert light into electrical energy (Mehdi et al. 2013). In 1943, Se was recognized to be a toxic and dangerous element causing serious poisoning (alkali disease) in livestock (Moxon and Rhian 1943). Later, however, it was discovered to be an essential element for animals (Schwarz and Foltz 1957). The necessity of Se for human and animal health was recognized when it was found to be a fundamental component of selenoenzymes such as glutathione peroxidase (GPX) and thioredoxin reductase (TR) (Rotruck et al. 1973; Sun et al. 1999).

The functions of selenoproteins in humans, animals, bacteria and algae are well known, but evidence for their presence in higher plants is still debated. Nevertheless, several studies have demonstrated various beneficial effects of low Se dosages in plants, including improved tolerance to different biotic or abiotic stresses (Hartikainen and Xue 1999; Xue and Hartikainen 2000; Hasanuzzaman et al. 2012; Qing et al. 2015) and promotion of growth and physiological functions (e.g., Xue et al. 2001; Djanaguiraman et al. 2005; Turakainen et al. 2006; Hajiboland et al. 2015). In fact, Se is taken to be a double-edged sword. Its optimal intake level for humans has a relatively narrow range between deficiency and toxicity (30 - 900 $\mu\text{g day}^{-1}$) (Fairweather-Tait et al. 2011). The daily Se intake by humans and animals mainly depends on its concentration in crops (food or feed) and ultimately on its concentration in agricultural soils (Govasmark and Salbu 2011).

Globally, Se is unevenly distributed in soils. Its concentration varies from less than 0.1 mg kg^{-1} , e.g., in Finnish podzol soils, to more than 80 mg kg^{-1} in the seleniferous soils of the western United States (Oksanen and Sandholm 1970; Gupta and Gupta 2000). In 1992, the Food and Agricultural Organization (FAO) reported that the lowest Se concentration (0.008 mg kg^{-1} DM) in cereals collected from different countries, was recorded in Finland (Sillanpää and Jansson 1992). In 1984, Finnish authorities decided to raise the Se concentration of food and feed by adding sodium selenate (Na_2SeO_4) to synthetic fertilizers (Eurola et al. 1990). The goal was to elevate the daily Se intake of Finnish people to the optimal range of 70 – 80 μg (Alfthan et al. 2015).

Nowadays, fertilizers are enriched with Se in several other countries such as Australia, New Zealand, the UK and Malawi (Wichtel 1998; Lee et al. 1999; Fordyce 2007; Broadley et al. 2006). Agronomic biofortification via application of mineral micronutrient fertilizer, to overcome the micronutrient deficiency in humans, is a way to increase the micronutrient concentration in edible parts of crops and hence daily intake by humans. Agronomic biofortification by inorganic Se fertilizers has successfully increased the daily Se intake in humans and animals in most countries suffering from Se deficiency such as Finland (Alfthan et al. 2015). However, the efficiency of agronomic biofortification and the recovery of Se added with fertilizers in crops is between 5 – 20 % (Ylärinta 1985; Broadley et al. 2010). The inorganic fertilizer Se dissolved in soil solution 1) accumulates onto the surfaces of Al and Fe

(oxy)hydroxides and 2) is taken up by plants and some of it is returned to the soil with plant residues (Yläranta 1985; Keskinen et al. 2011; Keskinen et al. 2013).

The Se accumulated in soil may be converted to immobile forms, leach from soil or volatilize to the atmosphere (Lyons 2010; Keskinen et al. 2013). Several chemical, physical and (micro)biological soil properties (e.g., pH, conductivity and activity of microorganisms) as well as plant species and forms of fertilizer Se dictate its fate and efficiency in the agroecosystem (Frankenberger and Karlson 1994; Lin et al. 2000; Banuelos et al. 2015). Long-term use of Se fertilizers can lead to excessive Se accumulation in soil and subsequently to pollution of nearby ecosystems (Miller and Welch 2013). In Finland, in a long-term field experiment from 1992 to 2004, the application of Se increased the Se concentration in both organic and recalcitrant soil fractions (Keskinen et al. 2011). This supports the assumption of Yläranta (1985) that residual fertilizer Se accumulates in soil in insoluble forms. The present study was undertaken 1) to unravel the potential bottlenecks in agronomic Se biofortification strategies and 2) to investigate the potential of Se-enriched plant residues as an additional source of Se for next growing season. This information is useful to find ways to overcome obstacles in Se biofortification and to reduce the potential risk of continuous inorganic Se fertilization in terms of agroecosystem contamination.

1.1. Selenium

1.1.1. Physicochemical properties of Selenium

Selenium is a metalloid in the group VIA (chalcogen) of the periodic table between sulfur (S) and tellurium (Te) and it has similar physicochemical properties to these two elements (Mehdi et al. 2013). It has different solid allotropic forms (amorphous and crystalline varieties) with semiconductor and photoconductor properties (Mehdi et al. 2013). Selenium is used widely in industrial products such as glasses, photovoltaic cells, plain-paper copiers and laser printers, alloys and X-ray devices (Fordyce 2007). It has six different stable isotopes with different abundance: ^{74}Se (0.87%), ^{76}Se (9.02%), ^{77}Se (7.58%), ^{78}Se (23.52%), ^{80}Se (49.82%) and ^{82}Se (9.19%) (Hoffmann and King 1997). Moreover, it has four different oxidation states: selenide (Se^{2-}), elemental selenium (Se^0), selenite (Se^{+4}) and selenate (Se^{+6}). In organic compounds, Se occurs as the amino acids selenomethionine (SeMet) and selenocysteine (SeCys) along with methylated forms such as dimethyl selenide (DMSe) (Seby et al. 2001).

1.1.2. Importance of selenium for human and animal health

Inadequate Se intake by humans and animals can result in many metabolic diseases and disorders, such as white-muscle disease and ill-thrift in calves, liver damage in pigs and exudative diathesis in chicks (Oldfield et al. 1960). The first recorded Se deficiency disorder in humans was Keshan disease, a cardiomyopathy in children and young women, in China (Tan et al. 1987). Many studies have shown that the Se deficiency causes a range of diseases such as heart disorders, weakened immune resistance to infections, male infertility, hypothyroidism and higher risk of different cancers (Abdulah et al. 2005; Roman et al. 2014). Selenium is an essential trace element for human and animal health because it is needed in the synthesis of selenoproteins. The recommended dietary allowance (RDA) of Se for humans is 55 –

400 $\mu\text{g day}^{-1}$ in the USA (National Academy of Sciences. Institute of Medicine. Food and Nutrition Board, 2000). There is a narrow distance between the beneficial effects of Se and its toxicity, but in contrast to Se deficiency, Se toxicity is rare and results in the loss of hair and nails, skin lesions, vomiting, nausea, anemia and chronic disorders such as hypertension and cardiovascular diseases (Huang et al. 2013).

1.1.3. Selenium in soils

The main sources of Se for humans are directly via plants or indirectly via animals fed on plants. The Se content in plants, in turn, is strongly correlated with its concentration in soil. Thus, any changes in the content and availability of Se in soil determine the Se daily intake by humans (Lokeshappa et al. 2012). The Se concentration in the earth's crust varies widely, being as low as 0.005 mg kg^{-1} in Finland and as high as 8000 mg kg^{-1} in Tuva-Russia (Bentley and Chasteen 2002; Oldfield 2002). In soils it is dictated by the type of geological parent material, atmosphere and anthropogenic activities. Typically, the Se concentration in soils ranges from 0.1 to 2.0 mg kg^{-1} (Dungan and Frankenberger 1999) but in seleniferous areas such as some parts of China, the USA, South America, Canada, India and Russia, it can reach 8000 mg kg^{-1} . In contrast, in New Zealand, the UK, Nordic countries (including Finland) and in some parts of China, the Se concentration is very low (less than 0.04 mg kg^{-1}) (Oldfield 2002; Hawkesford and Zhao 2007).

Soils formed from igneous rocks are low in Se concentration whereas soils developed from sedimentary rocks and associated with shales, sandstones, limestones and coal are higher in Se (Haygarth 1994; Fordyce 2007). In Finland, soils are formed from Precambrian bedrocks low in Se (0.025 – 0.1 mg kg^{-1}) (Koljonen 1975). The lowest Se concentrations (<0.01 mg kg^{-1}) are recorded in coarse mineral soils. In Finland, the Se concentration in organic soils is reported to be 0.18 – 0.22 mg kg^{-1} and in mull 0.09 – 0.12 mg kg^{-1} (Koljonen 1974; Sippola 1979). It is noteworthy that various Se fractions differ in their bioavailability, so the total Se concentration cannot be used in the estimation of Se bio-accessible reserves. Sequential extraction procedures (SEPs) have been used to identify various Se forms (Keskinen et al. 2011). In the SEPs, various Se species in soil are sequentially extracted by different solutions to provide information about the Se reserves differing in their bioavailability (Keskinen et al. 2011).

The atmospheric and anthropogenic activities are also important factors affecting the Se concentration in soils. For instance, volatile Se compounds originating from volcanic activity can be deposited from the atmosphere onto the land. In the UK, the estimate of annual atmospheric deposition of Se is 2.2 – 6.5 g ha^{-1} (Fordyce 2007). Moreover, anthropogenic activities such as fossil fuel combustion, metal industry and the use of fertilizers elevate the Se content in soils (Haygarth et al. 1993; Fordyce 2007). Se-enriched fertilizers are used in areas where Se deficiency needs to be overcome (Fordyce 2007). In Finland, the use of Se fertilization for 30 years (1984 – 2014) is taken to explain the 20% increase in total Se concentration in Finnish agricultural soils (Alftan et al. 2015).

The various Se species differ in their mobility, bioavailability and toxicity in the soils (Adriano 1986). The chemical forms of Se in soils are controlled by pH and redox

conditions. Selenate is the most soluble, mobile and bioavailable inorganic species in high redox conditions and alkaline soils where it is weakly bound onto soil particles (Alemi et al. 1988; Eich-Greatorex et al. 2007). Selenite is more stable and less bioavailable due to its high sorption tendency onto the surfaces of Al and Fe (oxy)hydroxides (Elrashidi et al. 1987; Saha et al. 2004). In reduced redox conditions, acidic and organic-rich soils, the major species are elemental Se, selenide and selenium sulfides. These species are less bioavailable to plants due to their low solubility and oxidation potential. They are strongly bound in organic compounds such as humic acids (Elrashidi et al. 1987). Microorganisms are important in the formation of organic Se species such as SeMet and SeCys or its mineralization in the soils (Seby et al. 2001). Methylated compounds are easily volatilized by microorganism or plants into the atmosphere (Terry et al. 2000; Sors et al. 2005).

Soils are complicated microenvironments differing in physical and chemical properties and microbiological activity. Thus, all the Se species can coexist and be transformed to each other and biological transformations, climate conditions and anthropogenic changes affect the Se cycling in the environment (Stolz et al. 2006; Chen et al. 2009).

1.1.4. Selenium in plants

As yet, there is no evidence that Se is necessary in higher plants (Terry et al. 2000; Fordyce 2013). Plants take up and assimilate Se into amino acids (SeMet and SeCys) by the same pathway as S (Brown and Shrift 1981; Terry et al. 2000). In general, plants differ in their ability to accumulate, assimilate and tolerate Se in their tissues. Most plants are non-accumulators that cannot accumulate Se in their tissues more than 10 – 100 $\mu\text{g g}^{-1}$ DW (White et al. 2004). Some *Allium* and *Brassica* species are Se indicators and accumulate it in their tissues up to 1 mg g^{-1} DW. The Se concentration in these plant tissues is directly related to the amount of bioavailable Se in soils. Some *Brassicaceae*, *Fabaceae* and *Asteraceae* species are hyper-accumulators containing Se in their tissues more than 1 mg g^{-1} DW. These plants typically grow in seleniferous soils (Terry et al. 2000). In addition, beneficial effects of low Se dosages on plant growth and their resistance to various abiotic and biotic stresses have also been reported (Lyons et al. 2009; El Mehdawi and Pilon-Smits 2012; Feng et al. 2013). However, an excessive Se accumulation in plants is toxic because incorporation of SeCys or SeMet into proteins impairs their functions (Brown and Shrift 1982; Van Hoewyk 2013).

1.1.4.1. Selenium uptake by plants

Plant roots take up both inorganic (SeO_4^{2-} , SeO_3^{2-}) and organic Se forms (SeMet and SeCys) from the rhizosphere soils, but not elemental Se (Shrift and Ulrich 1969; White et al. 2004; König et al. 2012). Plants readily take up SeO_4^{2-} from soils via high affinity sulfate-proton co-transporters, homologous to AtSULTR1;1 and AtSULTR1;2 transporters in *Arabidopsis thaliana* [L.] Heynh. (White et al. 2004; Sors et al. 2005). In rice (*Oryza sativa* L.), selenite as HSeO_3^- is taken up by phosphate transporters such as OsPT2 (Zhang et al. 2014) whereas H_2SeO_3 is taken up via the homologue of aquaporin channel, OsNIP 2;1 (Zhao et al. 2010; Pommerrenig et al. 2015). In *Arabidopsis*, different amino acid transporters such as LHT1, AAP1, AAP5 and ProT2 are known to transfer various amino acids from soil into the roots (Tegeger 2012).

Root cells probably use same amino acid transporters for SeMet and SeCys uptake (Tegeder 2012).

1.1.4.2. Selenium assimilation within plants

A summary of Se assimilation pathways in plants is shown in Figure 1. The assimilation of Se occurs in both cytosol and chloroplast (Pilon-Smits et al. 2010). Selenate is transferred by sulfate transporters (SULTR) into the cytosol of leaf cells and then it is translocated into the chloroplast via the homologue of *Arabidopsis* AtSULTR3;1 in the chloroplast membrane (Cao et al. 2013). First, selenate is reduced to selenite by two sequential enzymatic reactions including ATP sulfurylase (APS) and APS reductase (APR) via an intermediate product as adenosine phosphoselenate (APSe) (Pilon-Smits et al. 2010). Then, selenite is reduced non-enzymatically by reduced glutathione (GSH) or enzymatically by sulfite reductase (SiR) (González-Morales et al. 2017). Later it is coupled with O-acetylserine (OAS) by cysteine synthase to produce SeCys (Pilon-Smits et al. 2010). The conversion of SeCys to SeMet takes place in a sequential process. First, selenocystathionine is generated by cystathionine γ -synthase (CGS) and then selenohomocysteine (SeHCys) is produced by cystathionine β -lyase (CBL). Finally, SeHCys is converted to SeMet by methionine synthase (MTR) in the cytosol (Pilon-Smits et al. 2010).

Both SeCys and SeMet have multiple possible fates in plants. In addition to incorporation into proteins, they are methylated to volatile forms or converted to nonprotein amino acids (Pilon-Smits et al. 2010; White 2016 and 2018). In hyperaccumulator plants, SeCys is modified to SeMSeCys by SeCys methyl transferase (SMT). SeMSeCys is not incorporated into proteins and accumulates safely in these plants. SeMSeCys is further methylated to dimethyl diselenide (DMDSe), a major volatile compound in hyper-accumulator plants (Kubachka et al. 2007). SeMSeCys is also combined with glutamate via γ -glutamyl cysteine synthetase to produce γ -glutamyl-SeCys (GG-SeCys), a major storage form of Se in hyper-accumulator seeds (Freeman et al. 2007; Kubachka et al. 2007). Moreover, SeCys can be converted to elemental Se (Se⁰) by selenocysteine lyase (SL) in chloroplasts and mitochondria (Pilon et al. 2003). In the cytosol, SeMet is also methylated to MSeMet by methionine methyltransferase (MMT) and then it is further methylated to dimethyl selenide (DMSe) (White 2016 and 2018). Similar to SeCys, SeMet also can be converted to γ -glutamyl-SeMet (GG-SeMet) (White 2018).

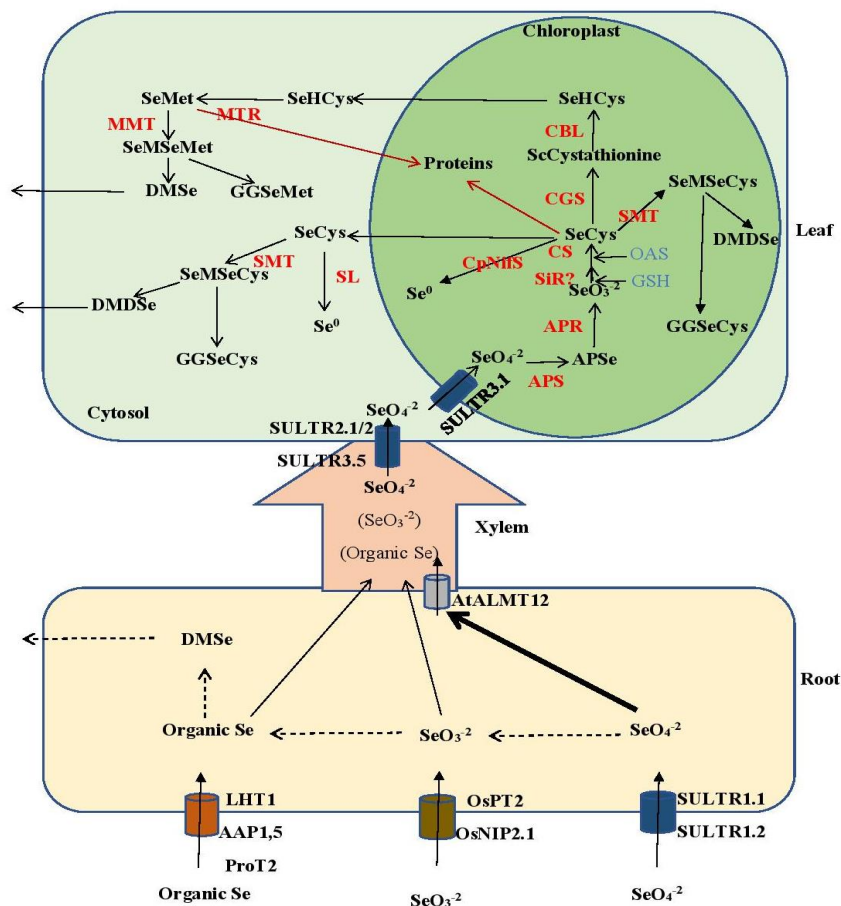


Figure 1. A scheme of Se uptake and its assimilation pathway in higher plants. All details are explained in the text. Abbreviations: AAP: amino acid permeases, APR: adenosine 5'-phosphosulfate reductase, APS: adenosine triphosphate sulfurylase, APS_e: adenosine 5'-phosphoselenate, AtALMT12: Aluminium activated malate transporter in *A. thaliana*, CBL: cystathionine β-lyase, CGS: cystathionine γ-synthase, CpNifS: Chloroplast SeCys lyase, CS: cysteine synthase, DMDSe: dimethyl diselenide, DMSe: dimethyl selenide, GGSeCys: γ-glutamyl-SeMSeCys, GGSeMet: γ-glutamyl-SeMet, GSH: glutathione, LHT: Lysine-Histidine like transporters, MMT: S-adenosyl-methionine:methionine methyl transferase, MTR: methionine synthase, OAS: O-acetylserine, OsNIP: Nodulin 26-like intrinsic proteins in *Oryza sativa*, OsPT2: phosphorus transporters in *Oryza sativa*, ProT: protein transporters, Se⁰: elemental Se, SeCys: selenocysteine, SeHCys: selenohomocysteine, SeMet: selenomethionine, SeMSeCys: Se-methyl selenocysteine, SeMSeMet: selenomethyl selenomethionine, SeO₃²⁻: selenite, SeO₄²⁻: selenate, SiR: sulfite reductase, SL: SeCys lyase, SMT: selenocysteine methyltransferase, SULTRs: sulfate transporters.

1.1.4.3. Physiological and antioxidative effects of Se on plant growth and development

Numerous studies have reported on the beneficial effect of Se on plant growth, development and functioning (Xue et al. 2001; Cartes et al. 2010; Chu et al. 2010; Feng et al. 2013; Hajiboland et al. 2015). Plants produce reactive oxygen species (ROS) during many metabolic processes in chloroplasts, mitochondria and peroxisomes (Elstner 1991), but they also eliminate ROS via enzymatic and non-enzymatic processes (Mittler 2002). When the production of ROS is higher than its scavenging, oxidative stress results, leading to dysfunction of DNA, proteins and lipids in the cells (Mittler 2002). Application of Se at low dosage was shown to increase plant tolerance under oxidative stress by reducing lipid peroxidation and improving the function of anti-oxidative enzymes (Xue et al. 2001). Therefore, Se plays a protective role in plants under various abiotic and biotic stresses including drought (Feng et al. 2013; Hajiboland et al. 2015), cold (Chu et al. 2010) and toxicity of soluble Al^{3+} and heavy metals such as Cd^{2+} (Issa and Adam 1999; Sieprawska et al. 2015).

Selenium application at 1, 2 and 3 mg kg⁻¹ soil was reported to significantly enhance the root growth, peroxidase and catalase activities in wheat seedlings, and under drought conditions the content of proline, carotenoids and chlorophyll (Hajiboland et al. 2015). Selenium has also been reported to alleviate the effect of cold on wheat seedlings by increasing the activity of the antioxidative system and chlorophyll content (Chu et al. 2010). Other studies have shown that Se application reduced the adverse effects of As in mung bean (*Vigna radiata* (L.) Wilcz.) (Malik et al. 2012), Cd^{2+} in oilseed rape (*Brassica napus* L. var. *oleifera* Metzg.) seeds (Sieprawska et al. 2015) and Al^{3+} in ryegrass (*Lolium perenne* L.) roots (Cartes et al. 2010).

The positive effects of Se on the growth and yield of various crops have been reported e.g., Indian mustard (*B. juncea* (L.) Czern.) (Singh et al. 1980), lettuce (*Lactuca sativa* L.) and ryegrass (Hartikainen et al. 1997, Hartikainen and Xue 1999) and soybean (*Glycine max* (L.) Merr.) (Djanaguiraman et al. 2005). Selenium is found to enhance the carbohydrate accumulation and storage quality of potato (*Solanum tuberosum* L.) tubers (Turakainen et al. 2006) along with photosynthesis rate and intercellular CO₂ concentration in rice plants (Zhang et al. 2014). However, in higher dosages Se acts as an oxidant which leads to oxidative stress probably through the imbalance in GSH, ferredoxins, thiol groups and NADPH levels in the cells (Hartikainen 2005).

1.2. Selenium biofortification and fertilization in Finland

Globally, areas with Se deficiency are more common than areas where the problem is a too high Se concentration (Haug et al. 2007). The options to improve the Se intake by humans and animals have been investigated in numerous studies (Euroala et al. 1990; Broadley et al. 2010; Seppänen et al. 2010; Boldrin et al. 2013; Duran et al. 2013; Banuelos et al. 2015). There are two main strategies, the first one being the direct use of Se supplements and the second one a food system (or agricultural) approach through plant breeding (genetic) and fertilization (agronomic) (Broadley et al. 2006). The most feasible way to introduce Se gradually and safely into the food chain is to use inorganic Se fertilizers (Lyons et al. 2004; Li et al. 2007). In Finland, the Se-containing

(as selenate) fertilizers came into general use in 1985. The earliest study on the application of Se-enriched fertilizer had been published already (Gissel-Nielsen et al. 1984).

In Finland, long-term continuous studies on the use of selenate or selenite as a component of soil fertilizers or foliar application on different crops were undertaken from 1982 to 1990 by Ylärinta (1990). Based on promising results, the Ministry of Agriculture and Forestry (1984) decided that the mineral fertilizers can be supplied with inorganic selenate to improve the Se intake by the Finnish population. The aim was to elevate the Se content in crops from 0.01 up to 0.1 mg kg⁻¹ DW, *i.e.*, to the level of the adequate daily Se intake by humans and animals (Oksanen and Sanholm 1970; Sippola 1979). Cereals products contribute about 20% of the daily Se intake of the Finnish population (Alfthan et al. 2010). Furthermore, Finland imports foreign cereals to mix with domestic grains during milling (Alfthan et al. 2010). Therefore, the Se content of in food or feed was not sufficient to provide an adequate daily Se intake for the Finnish population. In 1984, fertilizers amended with selenate were used by farmers at the third cut of forage grasses. After 1984, NPK and NK fertilizers in each growing season have been amended with selenate (Keskinen 2012).

In 1996, as well as NPK and NK, N fertilizers were also amended with selenate (Keskinen 2012). The Se dosage added to fertilizers has not been constant, as it is based on monitoring the average daily Se intake by animals and humans. The Se dosage in fertilizer has been changed as follows: 6 mg kg⁻¹ for grasses and 16 mg kg⁻¹ for cereals (1984 - 1990); 6 mg kg⁻¹ for all crops (1990 - 1998); 10 mg kg⁻¹ for all crops (1998 - 2007), and 15 mg kg⁻¹ since 2007 (Eurola et al. 2011). As a result, average daily intake of Se has increased from 25 - 30 µg (Varo and Koivistoinen 1980) to 80 µg (Eurola et al. 2011).

1.2.1. Factors affecting Se biofortification

The efficiency of Se fertilizer depends on many factors such as chemical speciation, fertilizer dosage, application method, time of application and the agroecosystem. Understanding the fate of applied Se in the soils is crucial for identifying and implementing an effective fertilizer policy to overcome Se deficiency.

1.2.1.1. Form and dosage of selenium

Investigations on the feasibility of Se species and its dosage for fertilization started in the 1960s (Ylärinta 1983; Gissel-Nielsen et al. 1984). Most studies focused on different salts of selenite and selenate in combination with N and P fertilizers. The effects of selenate and selenite application have been studied in many experiments in Finland, UK, Denmark and Germany (Ylärinta 1983; Bahnert 1987; Lyons et al. 2004; Broadley et al. 2006). Selenate salt is reported to increase the Se content in the crops 8 – 20 fold as compared with selenite (Bisbjerg and Gissel-Nielsen 1969; Hayes et al. 1987). Other researchers have suggested that soil properties such as pH are important in terms of the Se species used for biofortification (Ylärinta 1990). In England, in silt loam soils with alkaline pH, selenate is more efficient than selenite in enhancing the Se uptake, whereas in acidic soils it can easily leach, convert to reduced forms or volatilize into the atmosphere (Van Dorst and Peterson 1984).

Application of elemental Se as a slow- and long-term releasing fertilizer has been studied but its uptake by plants was less than 0.5% (Gissel-Nielsen 1970). Plants can take up organic Se species (mainly SeMet) up to 100 times faster than selenate. However, within plants organic Se is not as mobile as selenate (Shrift and Ulrich 1969; Sors et al. 2005; Kikkert and Berkelaar 2013). Stavridou et al. (2011 and 2012) reported that Se-enriched plant residues improved the Se uptake by catch crops. However, more studies are needed to test the possibility that Se-enriched plant residues could recycle as Se sources for crops.

1.2.1.2. Application time and method

In agricultural systems, an important aim is to minimize the number of fertilizer applications during the growing season. Different studies have reported that the half-life of selenate in a grassland ecosystem is short (21 to 70 days) (Watkinson 1983; Rimmer et al. 1990; Shand et al. 1992), so annual application of Se fertilizer is recommended. The short half-life of selenate in grassland may be attributed to the intensive nature of the cropping system and multiple harvests (three to five times) (Ros et al. 2016). Theoretically, there are two ways to optimize timing of fertilizer supply with crop demands. The first is the use of slow-release fertilizer at the level where crops can accumulate sufficient Se, which may decrease the loss of Se fertilizer in soil solution especially in areas with high environmental risk. The second option is frequent use of fast-releasing fertilizers. Since the efficiency of Se uptake is dependent on the time of application, a combination of both fast- and slow-release fertilizer strategies will probably give the best result (Ros et al. 2016).

Some studies have shown foliar application to be the most efficient method for Se fertilization. A foliar spray enhanced the Se uptake by crops two times more than the soil application (Hawrylak-Nowak 2009; Pezzarossa et al. 2012). The best time for foliar application is at the vegetative growth stage and before full leaf expansion (Ros et al. 2016). Other agronomic biofortification techniques include coating seeds or soaking them in a Se solution before sowing (Haug et al. 2007). TM minor differences were observed between seed coating, soil application or foliar spray of selenate on Se content of wheat plants, with slightly higher Se content in those plants treated with foliar spray at ear emergence time (Stephen et al. 1989). However, there is doubt that the seed treatment can be applicable to all crops and that it guarantees sufficient Se content (Ros et al. 2016). Moreover, application of Se with other nutrients such as N, P and S affects its uptake and accumulation in crops (Singh et al. 1980; White et al. 2004; Owusu-Sekyere et al. 2013; Zhang et al. 2014; Gonzales et al. 2017). These nutrients can compete with Se for uptake or enhance the Se retention in soil. In addition, through the resulting increase in yield, they can dilute Se content in crops (Lee et al. 2011; Dhillon and Dhillon 2000).

1.2.1.3. Rhizosphere bioactivity

The rhizosphere comprises the root and the volume of soil affected by biotic and abiotic factors (Turner et al. 2013). The accumulated Se in plants can be volatilized or end up back in soil with roots and litter material (Terry et al. 2000; Galeas et al. 2007; El Mehdawi et al. 2015). The selenoamino acids produced by plants serve as sources of N, C and Se for microbes (Terry et al. 2000). Bacteria convert inorganic Se into

organic Se compounds that will be volatilized as DMSe or DMDSe (Frankenberger and Karlson 1994; Zayed and Terry 1994; Winkel et al. 2015). Moreover, some bacteria reduce the selenate to selenite or elemental Se (Zayed et al. 1998; Lindblom et al. 2013; Winkel et al. 2015). Fungi can also reduce selenite to elemental Se, but do so less efficiently than bacteria (Wangelin et al. 2011; Lindblom et al. 2013).

Soil micro-organisms affect the growth of plants and nutrient accumulation in their biomass (de Souza et al. 1999; Wenzel 2009). Plants inoculated with appropriate bacteria are shown to accumulate more biomass and Se than non-inoculated ones (de Souza et al. 1999; Durán et al. 2013; El Mehdawi et al. 2015). In addition, in *B. juncea* and some aquatic species, Se uptake and volatilization are reported to increase in response to inoculation with bacteria isolated from Se-rich sediment (de Souza et al. 1999) or from the rhizosphere of Se-hyperaccumulator *Astragalus bisulcatus* (di Gregorio et al. 2005). Similar results were obtained with fungi instead of bacteria (Lindblom et al. 2013).

1.2.1.4. Climatic and temperature effect

In addition to soil properties, climatic conditions influence the Se uptake by crops (Haygarth et al. 1993; Galeas et al. 2007; Winkel et al. 2015). Thus, knowledge on both subjects is needed in order to optimize fertilizer strategies for areas of same soil properties (Spadoni et al. 2007). Variation in temperature and rainfall during the growing season affects soil moisture and pH (Cubadda et al. 2010) and hence the speciation and bioavailability of Se in soil. In a soil subjected to high precipitation, selenate is easily reduced to selenite that is strongly adsorbed onto the surfaces of Al or Fe (oxy)hydroxides (Hartikainen 2005). Hence, the concentration of bioavailable Se to be taken up by root plasma membrane decreases (Cubadda et al. 2010). High precipitation also promotes the leaching of Se from the bioavailable pool (Bitterli et al. 2010). On the other hand, dry weather reduces the Se uptake by plants as result of lowered water accessibility limiting the crop growth. Furthermore, high temperature promotes soil dryness and the oxidation of organic matter. Johnsson (1991) recorded a positive correlation between the Se content in wheat (*Triticum aestivum* L.) plants and the amount of precipitation during the growing period in Sweden.

1.3. The fate of Se fertilizer in the agroecosystem

Excessive use of chemical fertilizers leads to the accumulation of nutrients in the soil and, thus, to elevated environmental risks. However, in Se-deficient areas the amount of Se applied in fertilizers is low in terms of the bulk of soil volume. Thus, it is important to adopt appropriate fertilizing strategies that promote Se uptake and reduce the potential-environmental risks such as S accumulation in soils due to its competition with Se for root uptake. Moreover, global Se resources are limited and with the low recovery of Se fertilizers, there is concern that these resources will run out in less than 40 years (Haug et al. 2007; Ros et al. 2016). When choosing a proper fertilization strategy, it is fundamental to know the Se cycling in soils and within the plants. Thus, it is important to unravel the fate of fertilizer Se in the agroecosystems by using appropriate tools to integrate the complex interactions between soil properties, weather conditions, cropping systems and fertilizer management. The recovery of inorganic fertilizer Se is low (5- 30%), and most added Se fertilizer either

remains or will be returned to the soil with plant residues (Eich-Greatorex et al. 2007). The Se-enriched plant residues may act as a source of Se for plants and recycle Se in the agroecosystem. This potential source may, in turn, reduce the amount of chemical fertilizer required and the environmental risks. All this information can help the farmer to fit the biofortification strategy for any local conditions within and among agroecosystems.

2. AIMS OF THIS STUDY

The study mainly aimed to explore the fate of inorganic fertilizer Se added over 25 years to Finnish agricultural ecosystems for biofortification of crops. Based on previous results (Seppänen et al. 2010), most of the Se in fertilizers is not transferred to harvested parts of crops but its main part is either retained in soil or returned to soil in plant residues. Recycling of residual Se in soil and in the agroecosystem is still unknown. This study was undertaken to identify the bottlenecks in Se biofortification and to investigate the potential of Se-enriched plant residues to act as Se sources for crops in order to reduce the use of inorganic Se fertilizers. In the present study, oilseed rape plants that are secondary accumulators for Se were grown in greenhouse experiments and in the field along with wheat and forage grasses with the following specific objectives:

- a) to study the physiological effects of Se application (inorganic Se or Se-enriched plant residues) on plant growth and development;
- b) to investigate the potential of oilseed rape as an accumulator of bioactive and anticarcinogenic compounds such as SeMSeCys or other organic Se compounds in the seeds and meal;
- c) to examine the potential of Se in Se-enriched plant residues to be recycled and taken up by plants;
- d) to explore the role of soil micro-organisms on Se uptake and accumulation by plants;
- e) to identify the bottlenecks in Se biofortification pathways;
- f) to compare the efficiency of Se uptake and the recovery of fertilizer Se between different plant species; and
- g) to understand Se cycling in the sub-boreal field ecosystem.

3. MATERIALS AND METHODS

The experimental design and analytical methods of this PhD project are outlined below. More detailed information is presented in the original publications (I - IV).

3.1. Plant materials, greenhouse and field experiments

Two pot experiments (Exp1: 2010 and Exp2: 2012) were conducted under controlled conditions in the greenhouse of the Department of Agricultural Sciences of the University of Helsinki (60° 13' N, 25° 10' E). The field experiments (Exp3: 2011 and Exp4: 2012) were located at the Kotkaniemi experimental station of Yara Suomi Ltd. in southern Finland (60° 21' N, 24° 22' E). A summary of the experiments is shown in Table 1.

3.1.1. Greenhouse experiment 2010 (Exp 1)

Briefly, oilseed rape plants were grown in 1.5 l pots containing a sand: quartz sand (2.25:1) mixture. Selenium was applied to either the soil or the leaves. The Se treatments in the soil application were 0 and 130 $\mu\text{g plant}^{-1}$ as sodium selenate and those to the leaves were 0 and 30 $\mu\text{g plant}^{-1}$ as either selenate or selenite. Pots were arranged in a randomized complete block design (RCBD) with four replications in four blocks. Plants were harvested four times: 6 h, 7 d and 14 d after Se application and at the end of seed development. More details are presented in publication I.

3.1.2. Greenhouse experiment 2012 (Exp 2)

In the second greenhouse experiment, oilseed rape plants were grown in pots filled with sandy field soil high in organic matter. The source of inorganic Se was selenate applied at dosages of 0, 7 and 140 $\mu\text{g Se kg}^{-1}$ soil. The sources of organic Se were either non-enriched or Se-enriched leaf (17 g) and stem residues (21 g) which were mixed into the soil to give final additions of 0 and 7 $\mu\text{g Se kg}^{-1}$ soil. To study the effect of microbiological activity on the Se uptake by plants, autoclaved soil material with or without selenate application (0 and 7 $\mu\text{g Se kg}^{-1}$ soil) was used. Pots were arranged in an RCBD with four replications. Various plant parts were collected at rosette, inflorescence emergence, flowering and seed filling stages. Soil samples were collected at the end of experiment for Se analysis. More details are given in publications II and III.

3.1.3. Field experiment 2011 (Exp 3)

Wheat and oilseed rape were grown on the experimental fields at Kotkaniemi farm with Se applications of 0, 7.22 and 25 g ha^{-1} in the plots with four replicates of an RCBD. They were harvested at the rosette/tillering, inflorescence emergence, late flowering/heading and mature seed stages. The different plant parts were separated. At the end of experiment, soil samples were collected from the oilseed rape plots for Se analysis.

3.1.4. Field experiment 2012 (Exp 4)

In 2012, the grain crops were grown similarly as in 2011, except for an additional foliar application of N (20 kg ha^{-1}) after the rosette/tillering stage. Plants were harvested three times: at the rosette/tillering, late flowering/heading and mature seed stages. The plant parts were separated for Se analysis, and at the end of the experiment soil samples were collected from the oilseed rape plots. In addition, mixtures of forage

grasses containing timothy (*Phleum pratense* L.) and red clover (*Trifolium pratense* L.) were grown at three Se application levels: 0, 5.55 and 19.25 g ha⁻¹. They were harvested on 15 June and 16 August. All experiments were arranged in an RCBD with four replicates.

Table 1. Summary of greenhouse and field experiments (Exp 1 – Exp 4) conducted in this study.

| | Exp1 (greenhouse 2010) | Exp2 (greenhouse 2012) | Exp3 (field 2011) | Exp4 (field 2012) |
|------------------------------------|---|---|---|--|
| Plant species | <i>Brassica napus</i> L. | <i>Brassica napus</i> L. | <i>Brassica napus</i> L. <i>Triticum aestivum</i> L. | <i>Brassica napus</i> L. <i>Triticum aestivum</i> L. <i>Phleum pratense</i> L. <i>Trifolium pratense</i> L. |
| Form and amount of added Se | a) <i>Soil application:</i> Se in selenate (VI): 0 and 130 µg plant ⁻¹ b) <i>Foliar application:</i> Se in selenate (VI) and selenite (IV): 0 and 30 µg plant ⁻¹ | Se in selenate (VI): 0, 7 and 140 µg plant ⁻¹ Se in leaf residues: 0 and 7 µg plant ⁻¹ Se in stem residues: 0 and 7 µg plant ⁻¹ | Se in selenate (VI): 0, 7.22 and 25 g ha ⁻¹ | a) <i>Grain crops:</i> Se in selenate (VI): 0, 7.22 and 25 g ha ⁻¹ Foliar N: 0 and 20 kg ha ⁻¹ b) <i>Forage grasses:</i> Se in selenate (VI): 0, 5.55 and 19.25 g ha ⁻¹ |
| Plant sampling | 6 h, 7 d, 14 d after Se application and at seed maturity | Rosette, inflorescence emergence, flowering and seed filling stages | Rosette, bolting, flowering and seed filling stages | a) <i>2011</i> Rosette/tillering, inflorescence emergence, late flowering/heading and seed maturity stages b) <i>2012</i> grain crops: Rosette/tillering, late flowering/heading and seed maturity stages. Forage grasses: 15 June and 16 August |
| Soil sampling | no sampling | End of the experiment | End of the experiment | End of the experiment |
| Publication | I | II and III | IV | IV |

3.2. Methodology

The analyses and the analytical methods used in various experiments are summarized in Table 2.

Table 2. A summary of the methods used in this work. More details are given in the original publications (I-IV).

| Methods | Publications |
|---|--------------|
| Dry matter accumulation | I, II |
| Photosynthesis measurement by LI-6400, LI-COR | II |
| Leaf area measurement by LI-3000A, LI-COR | II |
| Chlorophyll content measurement by SPAD | I, II |
| Soil electrical conductivity | II |
| Soil pH | II |
| Soil respiration by GC | II |
| Selenium analysis in plants materials by AAS | I |
| Selenium analysis in plants materials by ICP | II, III, IV |
| Selenium analysis in soil materials by ICP-OES* | III, IV |
| Se speciation by HPLC-ICP-MS | I |
| Gene expression analysis by qPCR | I |
| Gene sequencing | I |

*This analysis was not carried out by NE

3.2.1. Measurement of plant growth parameters

In Exp1 the chlorophyll content of the young leaves was measured indirectly by SPAD-502 chlorophyll meter (Minolta Co. Ltd.) and in Exp2 directly by extracting with 80% acetone. Photosynthesis rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$) in the young and expanded leaves (fourth or fifth) was measured by LI-6400 (LI-COR Inc., Lincoln, NE, USA). After cleaning, the different plant parts were dried in an oven at 70°C for 48 h and weighed.

3.2.2. Soil analyses

3.2.2.1. Soil pH, electrical conductivity

Soil samples collected in the greenhouse (Exp2) and field experiments (Exp3, Exp4) were stored at +4°C until further analysis. A suspension of soil to water ratio of 1:2.5 (v/v) was used to measure soil pH and electrical conductivity (mS cm^{-1}).

3.2.2.2. Soil respiration measurement by GC

Soil samples from greenhouse experiment (Exp2), autoclaved and non-autoclaved treatments with or without inorganic Se application (0 and 7 $\mu\text{g kg}^{-1}$ soil) were chosen for measurement of soil respiration. Soil samples of 65 g with 60% WHC (water holding capacity) were weighed into jars (470 ml), sealed and incubated at 20°C for 24 h. Air samples from each jar were collected into vials and analyzed by gas chromatography.

3.2.2.3. Selenium analysis in soil samples by ICP-OES

At the end of the experiments, the soil samples collected from the oilseed rape plots in Exp2, Exp3 and Exp4 were used to determine their inorganic and organic Se pools. The inorganic Se adsorbed onto Al and Fe oxide particles was extracted by $\text{KH}_2/\text{K}_2\text{HPO}_4$ solution. After the suspension was centrifuged, the soil pellets were further extracted with NaOH to obtain the organic Se pool. Both inorganic and organic Se fractions were analyzed by ICP-OES in Metropolia lab (Finland).

3.2.3. Plant analyses

In all experiments, the plant parts collected at different harvesting times were cleaned and dried for Se analysis. In Exp1, total Se was analysed by electrothermal atomic absorption spectrometric (AAS) and in Exp 2, Exp3 and Exp4 by inductively coupled plasma - optical emission spectrometry (ICP-OES). In Exp1 the speciation of Se of plant samples was determined by HPLC-ICP-MS (high performance liquid chromatography - inductively coupled plasma - mass spectrometry) in Complutense University of Madrid, Spain.

3.2.3.1. Selenium analysis in plant samples by AAS

An acidic mixture of HNO_3 , HClO and H_2SO_4 was added to ground plant samples and incubated overnight in the digester (2040 Digester). To reduce all Se to SeIV , chelating and extracting reduced Se for analyzing by AAS (HGA 600, PerkinElmer), HCl , ammonium pyrrolidine dithiocarbamate and methyl isobutyl ketone were used sequentially. Two flour samples with known Se content were used as a house reference sample.

3.2.3.2. Selenium analysis in plant samples by ICP- OES

As above, plant samples were prepared but after the reducing step with HCl , samples were subjected to Se analysis by ICP-OES (iCPA 6000 Series, Thermo Scientific). The same standards as above were used for checking the accuracy of the results.

3.2.3.3. Selenium speciation in plant samples by HPLC-ICP-MS

Briefly, plant samples were incubated with Protease XIV and sonicated in a Sonoplus ultrasonic homogenizer (Bandelin Electronic GmbH & Co. Germany). After being filtered, the samples were analyzed by HPLC-ICP-MS. To identify organic Se, two HPLC columns, PRP X-100 anion exchange column and Shodex Asahipak, in combination with size-exclusion and ion exchange separation mechanism were used. Standard solutions of SeMet , SeMSeCys , SeCys (Sigma) and Na_2SeO_3 and Na_2SeO_4 (Merck) were prepared to check the accuracy of method.

3.2.4. Molecular analyses

Total RNA from the leaf samples of oilseed rape plants from Exp1 were extracted by TRI REAGENT (Applied Biosystem, Foster City, USA) according to the manufacturer's instructions. The cDNA was then synthesized by Super Script™ III reverse transcriptase (Invitrogen) according to the manufacturer's instructions. The q-RT-PCR step was undertaken in 96-well plates using the SYBG Green-based PCR assay in a Roche light cycler (Roche Applied Science, Basel, Switzerland). The relative expression level of target genes (*ASP1*, *ASP2*, *ASP3* and *SMT*) was calculated by the $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen, 2001) based on actin as the reference gene. The details are given in Publication I.

3.2.5. Index calculation

- A) Conductivity index = $10 \text{ (cm mS}^{-1}) \times \text{Electrical conductivity (mS cm}^{-1})$
- B) Seed translocation factor (SeedTF) = $\text{Se content}_{\text{seed}} / \text{Se content}_{\text{shoot}}$
- C) Translocation factor (TF) = $\text{Se content}_{\text{shoot}} / \text{Se content}_{\text{root}}$
- D) Selenium uptake efficiency (SeUPE) = $(\text{Total Se content}_{\text{treated}} - \text{Total Se content}_{\text{control}}) / \text{Se}_{\text{applied}}$
- E) Se fertilizer recovery (SeFR) = $(\text{Seed Se content}_{\text{treated}} - \text{Seed Se content}_{\text{control}}) / \text{Se}_{\text{applied}}$

3.2.6. Statistical analyses

In all publications (I-IV), data were grouped based on harvesting time and subjected to one-way analysis of variance (ANOVA) with comparison of means by Tukey's HSD test with $P \leq 0.05$. Data obtained from high Se application in publication II due to normalization of data was excluded from statistical analysis and was reported based on average. Multivariate analysis was conducted to test the interactions between soil and leaf application of Se (publication I) and between Se fertilizer levels and plant species (publication IV). SPSS (version 20) software (IBM Inc., Chicago, IL, USA) was used for all statistical analyses.

4. RESULTS AND DISCUSSION

4.1. Physiological effects of Se application

4.1.1. Selenium species and dosage affect biomass accumulation and growth parameters

Application of inorganic Se resulted in transiently higher dry matter accumulation and faster development in the vegetative phase (Figure 2). In Exp2, all Se applications ($7 \mu\text{g plant}^{-1}$), except Se-enriched stem residues, significantly increased the seed yield (Paper II, Ebrahimi et al. 2015, Figure 1). In addition, amending soil with stem residues slightly decreased the dry matter accumulation and retarded the phenological development at earlier vegetative phase. Similarly Trinsourtrot et al. (2000) showed that amending soil with *B. napus* residues resulted in a rapid C-substrate decomposition and immobilization of bioavailable N and, thus, a lower dry matter accumulation. At the same phase, the photosynthesis rate was slightly higher in the plants grown with Se application (Paper II, Ebrahimi et al. 2015, Figure 1 and Table 2).

Although Se is not proved to be essential for higher plants, its promoting effect on the plant growth and development has been frequently reported (Hartikainen et al. 2000; Turakainen et al. 2006; Hajiboland and Amjad 2007; Hawrylak-Nowak 2013; Hajiboland et al. 2015). Several studies have reported positive effects of Se application on the dry matter accumulation and yield in wheat (Nawaz et al. 2015), rice (Zhang et al. 2014), maize (Wang et al. 2012) and potato (Turakainen et al. 2004). Owusu-Sekyere et al. (2013) reported that Se treatment enhanced the carbohydrate accumulation and elevated the chlorophyll content in alfalfa (*Medicago sativa* L.) plants without any effect on the dry matter accumulation. They also suggested that the higher carbohydrate accumulation might be attributable to an increased net CO_2 assimilation and chlorophyll *a* content.

In field conditions (Exp3, Exp4), the addition of Se did not significantly affect the crop yield. Hajiboland et al. (2015) reported that application of selenate ($15 \mu\text{g l}^{-1}$) in the greenhouse and in the field (20 and 60 g ha^{-1}) increased photosynthesis rate and shoot biomass in both drought-sensitive (Sara) and -tolerant (Homa) wheat cultivars. Boldrin et al. (2013) showed that under greenhouse conditions, the foliar application of selenate or selenite ($50 \mu\text{g l}^{-1}$) to rice plants did not affect shoot dry mass, but increased grain yield. A soil application of selenate (0.75 mg kg^{-1}), in contrast, increased both the shoot dry matter and grain yield (Boldrin et al. 2013). The beneficial effect of low Se dosages on the plant growth parameters, especially under abiotic stress conditions, could be related to the scavenging of ROS such as $\text{O}_2^{\cdot-}$ and H_2O_2 and to increased antioxidative capacity of plants tissues (Hartikainen and Xue 1999; Pilon-Smits et al. 2009; Djanaguiraman et al. 2010). Selenium compounds may directly interact with ROS or induce the activity of antioxidative enzymes (Hajiboland and Amjad 2007; Djanaguiraman et al. 2010). However, the mechanisms behind these complex processes are not fully understood. Conversely, application of high Se can result in dysfunction of proteins incorporation of selenoamino acids (Hajiboland and Amjad 2007).

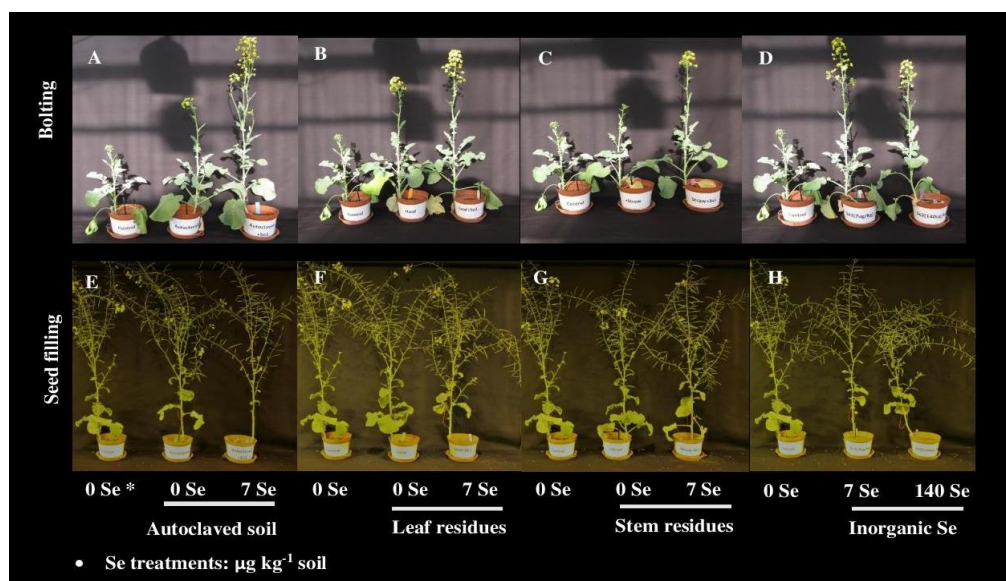


Figure 2. The growth of oilseed rape plants under different Se treatments and their development at bolting (first row) and seed filling (second row) stages. A and E: control plants and those grown in autoclaved soils without or with selenate application. B and F: control plants and those grown in soil amended with leaf residues or Se-enriched leaf residues. C and G: control plants and those grown in soil amended with stem residues or Se-enriched stem residues. D and H: control plants and those grown in soil fertilized with selenate dosages of 7 and 140 $\mu\text{g Se kg}^{-1}$ soil.

4.1.2. The effect of selenate or selenite applications on activity of Se assimilation genes and Se speciation within *B. napus* plants

ATP sulfurylase (APS), one of the key enzymes controlling Se accumulation in plants, converts selenate and ATP into adenosine phosphoselenate (APSe) in the chloroplasts (Terry et al. 2000; Sors et al. 2005). In this study, when selenate or selenite was added to soil or to leaves, the expression levels of *APS1-3* genes at 6 h, 7 d and 14 days after Se treatments were not significantly different (Paper I, Seppänen et al. 2018, Figure 1 a-c). However, the combination of soil and foliar application increased the transcript levels of *ASP 1,2* above the levels obtained with foliar application alone as selenate or selenite (Paper I, Seppänen et al. 2018, Figure 1 a-c). Selenate was the main accumulated species in the leaves 6 h after its application to soil (Paper I, Seppänen et al. 2018, Figure 3 and Table 1). Selenate accumulation in the leaves decreased after 14 days (from 81% to 60%), while selenite was detected after 7 days. This result is coincident with the elevated expression of *ASPs* genes (Paper I, Seppänen et al. 2018, Figure 1 and Figure 2). Previous studies have found that selenate is the most accumulated compound in non-accumulator plants (Terry et al. 2000; Sors et al. 2005). Another study has also shown that ASP is a key enzyme in selenate reduction and accumulation of Se in plants (Burnell 1981). Its overexpression in transgenic plants is found to result in higher Se accumulation in their tissues as compared with wild types (LeDuc et al. 2006).

The accumulation of SeMet in the leaves after 6 h was higher when Se was supplied as selenite rather than selenate (Paper I, Seppänen et al. 2018, Table 1). This is consistent with a faster assimilation of selenite into organic forms (Dumont et al. 2006). Moreover, in this study SeMet was the major organic Se species, representing up to 33% of total Se in the leaves and up to 96% in seeds and meal of oilseed rape plants (Paper I, Seppänen et al. 2018, Table 1 and Table 2). Similar results have been obtained with other plant species, such as Brazil nut (*Bertholletia excelsa* Humb. & Bonpl.), wheat, rye (*Secale cereale* L.), and barley (*Hordeum vulgare* L.) along with previous studies on oilseed rape (Stadlober et al. 2001; Dumont et al. 2006; Zhu et al. 2009; Seppänen et al. 2010; Poblaciones et al. 2014).

SeMSeCys, a major non-toxic Se compound in hyperaccumulator plants, was detected in the leaves only 6 h after Se treatment (Paper I, Seppänen et al. 2018, Figure 3 and Table 1). In the current study the amino acid sequence of SMT enzyme was very similar to that of homocysteine S-methyltransferase (HMT). These results suggest a dual HMT/SMT function of this enzyme in *B. napus* plants.

4.2. Agronomic biofortification

4.2.1. Selenium uptake by plants

The results of the greenhouse and field experiments revealed that the uptake and distribution of Se as well as its redistribution or remobilization in the plant depended on the plant species, Se species and its dosage, soil properties (e.g. pH and conductivity) and seasonal growth conditions. Total plant Se content in the whole plant increased at its higher dosages (Figure 3, Paper IV, Ebrahimi et al 2019, Figure 2) as shown in previous studies (Seppänen et al 2010; Srikanth-Lavu et al. 2013; Keskinen et al. 2013). In the greenhouse experiment, the plant Se content tended to increase with plant development, indicating continued uptake (Figure 3). The only exception was the flowering stage, when the plants Se content decreased in the plants treated with inorganic Se (Figure 3). This result was probably attributable to either the dilution of Se by a greater dry matter accumulation or a higher Se volatilization during the flowering stage. In the field, where it was not possible to collect senescing leaves, the plant Se content was lower at the late flowering stage in both oilseed rape and wheat plants (Paper IV, Ebrahimi et al 2019, Figure 2 a, b). The Se content of the forage grasses at second harvesting was lower than the first one due to the lower amount of weakly bound Se left on the sorption sites (Paper IV, Ebrahimi et al 2019, Figure 2 c).

In the plants supplied with selenate in the greenhouse experiment, the Se accumulation was 2 - 14 times higher than in those supplied with Se-enriched plant residues (Figure 3). In addition, in Exp1, Se speciation result showed that selenate was the most accumulated form in the leaves of *B. napus* plants 6h after selenite or selenate applications (Paper I, Seppänen et al. 2018, Table 1). These results agree with concept that selenate is the most mobile Se species in soils and plants (White et al. 2004; Sors et al. 2005). The translocation factors (TF, Figure 4) showed that the Se applied as selenate was more mobile within plants, with more translocated from root to shoot, than that taken up from Se-enriched leaf and stem residues. Kikkert and Berkelaar (2013) showed that the mobility of different Se species in oilseed rape and wheat plants decreased in the order: selenate > SeMet > selenite/SeCys. The high mobility of

selenate is attributable to its higher solubility in water and transporters activity for uptake from soil (Feist and Parker 2001; Zhang et al. 2003) and to its translocation within the plants by different sulfate transporters (White et al. 2004; Sors et al. 2005).

The rate of xylem loading is an important factor in the translocation of ions or molecules from root to shoots (Renkema et al. 2012). Selenate has a higher xylem loading capacity than selenite or organic Se species, because it has different types of transporters. Presumably the lower Se uptake in the plants treated with leaf or stem residues is attributable to their slow decomposition in the soil. Speciation of Se in oilseed rape plants by ion-pairing LC-ICP-MS has revealed that most of the Se accumulated in shoots is in the forms of selenate, SeMSeCys and SeMet (Dumont et al. 2006). Thus, the decomposition process of Se-enriched plant residues can explain their lower Se uptake by plants. Similarly, Ajwa et al. (1998) and Stavridou et al. (2012) reported that amending the soil with plant residues resulted in a lower Se accumulation in plants. This confirms the concept that the Se-enriched plant residues do not promote its uptake as efficiently as inorganic Se species. Furthermore, in the present study the selenate uptake was higher in the autoclaved soil than in the non-autoclaved one (Figure 3) and also promoted the plant growth and dry matter accumulation. Soil heating and autoclaving is reported to cause cell lysis of micro-organisms and hence the release of organically bound nutrients into the soil solution. These processes improve the nutrient status in the soil (Dunn et al. 1979; Kutiel and Shaviv 1989; Serrasolses et al. 2008).

In addition to the factors discussed above, the weather in the growing season affected the Se uptake by plants (Paper IV, Ebrahimi et al 2019, Figure 2). The cool, damp conditions during the growing season in 2012 were associated with a strongly reduced Se uptake being below the level recorded in 2011 (Paper IV, Ebrahimi et al 2019, Figure 1, 2). Changes in temperature and rainfall are reflected in soil properties such as e.g. electrical conductivity (Cubadda et al. 2010). According to Hartikainen (2005), a higher water content in the soil favours the reduction of selenate to selenite that has a higher sorption tendency onto the surfaces of poorly crystalline Al and Fe (oxy)hydroxides.

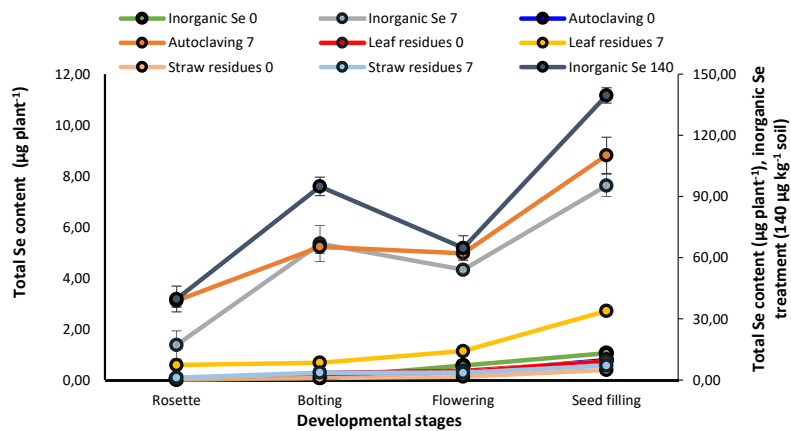


Figure 3. Dynamics of Se uptake ($\mu\text{g plant}^{-1}$) at rosette, bolting, flowering and seed filling stages of *B. napus* plants grown in soil treated with inorganic Se (0, 7 and $140 \mu\text{g kg}^{-1}$ soil), organic Se (leaf and stem residue, 0 and $7 \mu\text{g kg}^{-1}$ soil) and in autoclaved soil with inorganic Se (0 and $7 \mu\text{g kg}^{-1}$ soil). Data are expressed as means \pm SE, $n=3$.

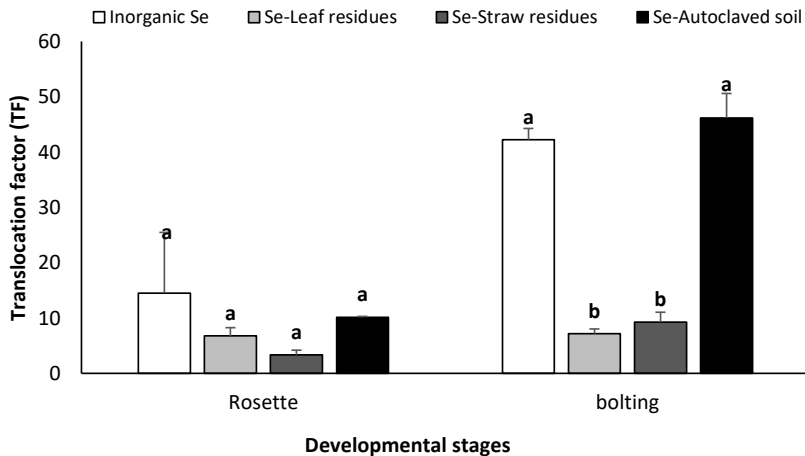


Figure 4. Translocation factor (TF) as ratio of Se content of shoot to root at rosette and bolting stages of *B. napus* plants grown in soil treated with inorganic Se ($7 \mu\text{g kg}^{-1}$ soil), Se- enriched leaf and stem residue ($7 \mu\text{g kg}^{-1}$ soil) and in autoclaved soil with inorganic Se ($7 \mu\text{g kg}^{-1}$ soil). Data are expressed as means \pm SE, $n=3$. Within a developmental stage, bars with different letters are significantly different by Tukey's HSD test ($P < 0.05$).

4.2.2. Distribution and remobilization of Se

There are numerous studies on Se uptake and its assimilation pathways, but few have focused on Se remobilization during plant development. The efficiency of nutrient use depends on their uptake from the soil and their further delivery into the harvestable plant parts such as seeds. This nutrient uptake process includes several steps: uptake from soil, translocation, remobilization and storage. Thus, in order to improve the biofortification strategies in plant production, it is important to understand the relative contribution and coordination of these steps.

The results of Exp2 showed that the uptake of Se by the plants depended on its dosage and species as well as on the developmental stage of the plants (Paper II, Ebrahimi et al. 2015, Tables 3-6). At all developmental stages, inorganic Se produced higher Se concentrations in various plants parts than did the Se-enriched plant residues (Paper II, Ebrahimi et al. 2015, Table 3-6). Moreover, during the maturation of the plants most of the Se accumulated in the aerial parts and later in the reproductive parts (Paper II, Ebrahimi et al. 2015, Table 3-6).

The results of Se distribution in various plants parts (Paper II, Ebrahimi et al. 2015, Table 3-6; Paper III, Ebrahimi et al. 2019, Table 1) and its total content in plants (Figure 3) suggest that Se content of root, stem and leaves generally are the most variable parts during plant development. Therefore, it can be supposed that during senescence Se will be remobilized from the leaves, roots and stems into the seeds. The current study and the previous ones (Seppänen et al. 2010; Banuelos et al. 2012) reveal that inorganic Se (selenate or selenite) in the leaves and SeMet (up to 96% in our results) in the seeds are the main accumulated Se species (Paper I, Seppänen et al. 2018, Table 1 and Table 2). Se is assimilated and incorporated into amino acids and proteins by the S pathway (Terry et al. 2000; Sors et al. 2005). Silique walls of oilseed rape plants are considered to be the sites for biosynthesis of S-containing compounds from the precursors from vegetative plant parts (Bloem et al. 2007). It can be speculated that Se is loaded from silique walls into the seeds by the same mechanism as S. However, our results revealed that the Se content in the seeds is restricted more by its loading from the silique walls rather than by its remobilization during senescence. These results showed that selenate is the most bioavailable Se species in soil and most mobile within plants as compare with Se-enriched residues. On the basis of the present study, two bottlenecks in the Se biofortification pathway can be proposed. The first one is the bioavailability of Se in the soil for root uptake and the second one is its remobilization and translocation into the plant parts to be harvested.

In 2012, when the conditions in the growing season limited the Se uptake, its proportion in the reproductive parts was higher than in the stems or roots (Paper IV, Ebrahimi et al. 2019, Table 5). Furthermore, the concentration of Se in the grains of wheat was higher than in the seeds of oilseed rape (Paper IV, Ebrahimi et al. 2019, Table 4 and Table 5). In addition, in oilseed rape plants, the remobilization of N during senescence is weaker than in wheat and barley (Avice and Etienne 2014; Maillard et al. 2015; Etienne et al. 2018). During senescence, wheat plants have been shown to remobilize N and micronutrients (Cu, Zn and Fe) more than oilseed rape does (Uauay et al. 2006; Kutman et al. 2011; Waters and Sankaran 2011). Moreover, Bianga et al.

(2013) indicated that 80% of proteins stored in wheat grains are as glutenin and gamma-gliadin that are rich in Met and Cys residues. Selenium can substitute for some S in these amino acids, which was shown in the Se content of the wheat grains being higher than in the oilseed rape seeds. In the field experiment in 2012, N application clearly enhanced the remobilization of Se from the leaves into the seeds (Paper IV, Ebrahimi et al. 2019, Table 5). Since N and Se are assimilated to organic compound as amino acids, thus application of N can improve the assimilation rate of Se which are remobilized into the sink organs like grains and seeds (Ramkissoo et al. 2019).

4.2.3. Recovery of fertilizer Se is affected by its chemical species, plant species and seasonal growth conditions

In 2011 the Se concentration in the seeds of the oilseed rape grown at a low Se level both in the greenhouse ($7 \mu\text{g kg}^{-1}$ soil, Paper III, Ebrahimi et al. 2019, Figure 1) and in the field (7.2 g ha^{-1} , Paper IV, Ebrahimi et al. 2019, Table 2) reached the level recommended by Eurola et al. (1990) for Finnish cereals ($0.1 - 0.2 \text{ mg kg}^{-1}$ DW). However, in 2012, only the high Se dosage was able to elevate the Se concentration in the seeds to an adequate level. Interestingly, the wheat grains had the same Se concentration as the oilseed rape seeds, although their total Se uptake was lower (Paper IV, Ebrahimi et al. 2019, Table 2 and Figure 2).

The applied Se-enriched plant residues did not increase the Se concentration in the seeds due to the low bioavailability of Se in the residues. Thus, the potential of the Se-enriched plant residues to recycle Se and to promote its uptake by plants is limited. However, in the autoclaved soil amended with inorganic Se its concentrations in the seeds of oilseed rape were significantly higher than in the non-autoclaved ones (Paper III, Ebrahimi et al. 2019, Figure 1).

As for the forage grasses, at both harvestings the Se concentration in the shoots was significantly higher in the timothy than in the red clover (Paper IV, Ebrahimi et al. 2019, Table 3). However, the cuts did not markedly differ in terms of the Se concentration in the shoots (Paper IV, Ebrahimi et al. 2019, Table 3).

In the grain plants, the recovery of fertilizer Se ranged from 0.5 to 16% depending on the dosage and Se species, plant species and growing season conditions. In the grass, the recovery of fertilizer Se was between 6 and 62% depending on the Se dosage and the time of harvest. This recovery, despite unfavorable growing conditions in 2012, was higher than it for grain crops (Paper III, Ebrahimi et al. 2019, Figure 3, Paper IV, Ebrahimi et al. 2019, Table 6, 7). As discussed in the previous sections, all the factors affecting the Se uptake (Se dosage and species, plants species, soil properties and growing weather) also affect the recovery of fertilizer Se.

4.3. Accumulation of Se in the soil

Analysis of inorganic and organic Se pools in the soil samples collected in the greenhouse experiment revealed that autoclaving of soil significantly reduced the inorganic Se pool and slightly also the organic pool (Paper III, Ebrahimi et al. 2019, Figure 4). This result agrees with the Se accumulation being higher in the plants grown in the autoclaved soils (Paper III, Ebrahimi et al. 2019, Table 2 and Figure 3). Heating releases Se from its sorption sites (Serrasolses et al. 2008). It can also result in the

lysis of soil micro-organism cells, making the nutrients bioavailable (Dunn et al. 1979; Kutiel and Shaviv 1989). In addition, autoclaving can kill the soil micro-organisms, which prevents Se volatilization and their competition with plants for Se. These processes can explain the higher Se accumulation in the plants grown in the autoclaved soils (Paper III, Ebrahimi et al. 2019, Table 2 and Figure 3).

The long-term field study indicated that a high dosage of NPK fertilizer (160 kg ha^{-1}) elevated the organic Se pool both in topsoil (0.00 – 0.05 m) and subsoil (0.30 m) (Paper IV, Ebrahimi et al. 2019, Figure 3c). As the results of the greenhouse experiment revealed, the Se-enriched plant residues in soil are not easily bioavailable. Their gradual accumulation in soil during long-term cultivation explains a slow increase in the organic Se pool in the field soils.

The short-term study revealed that applied Se did not significantly affect the Se concentration in soil, due to the small amount of applied Se fertilizer and its dilution in the bulk of soil (Paper IV, Ebrahimi et al. 2019, Figure 3 a and b). In their study on 10 different fields, Stroud et al. (2010) reported that Se fertilization in all fields except one, had no effect on the total Se concentration in soil after harvesting of crops. This is likely because selenate can easily leach down in the soil profile (Stroud et al. 2010) and is also volatilized by rhizosphere micro-organisms (Lin et al. 2000). The increase ($4 \mu\text{g Se kg}^{-1}$) of extractable Se from the topsoil sample was related to the field fertilized with 50 g Se ha^{-1} (Stroud et al. 2010). Soil from this field was high in clay, with moderate carbon content and low pH which are favorable for absorbing Se into soil particles (Stroud et al. 2010).

4.4. The fate of fertilizer Se in the field ecosystem

In Finland since 1984, selenate has been added to the fertilizers to elevate the Se concentration in the crops and to promote the daily Se intake by the Finnish population (Alfthan et al. 2015). The amounts of Se in human and animal serums have been continuously monitored to prevent Se toxicity and to adjust the amount of Se to be applied in fertilizers if the need arises (Keskinen 2012). Monitoring of the fate of applied Se in agroecosystems is an important issue in terms of biofortification strategies and environmental risks of Se accumulation in soil (Ros et al. 2016). In the current work, oilseed rape was used as a model plant to develop a schematic model for Se cycling in the agroecosystems. The results indicated that, depending on climate conditions, only up to 45% (2011) or 7% (2012) of added Se was taken up by oilseed rape plants (Paper IV, Ebrahimi et al. 2019, Figure 4). Only up to 14% enters the food chain while the rest remains in the shoots (up to 30%) and roots (less than 2%) (Paper IV, Ebrahimi et al. 2019, Figure 4). This portion is returned to the soil with plant residues.

Our hypothesis to recycle the Se-enriched plant residues back to the soil in order for them to be uptaken again by plants failed due to the low bioavailability of organic Se species. It can be supposed that they accumulate in the soils, which explains the increases in the organic Se pools after long-term use of fertilizer Se in Finnish agroecosystems. In our model based on results reviewed by Winkel et al. (2015), 5-15% of applied Se is volatilized by plants or micro-organisms. All this information was used

to create a flow chart of Se cycling in the agroecosystem ((Paper IV, Ebrahimi et al. 2019, Figure 4).

5. CONCLUSIONS AND FUTURE PERSPECTIVES

Se hyperaccumulator plants can accumulate high concentrations of Se in their tissues due to the production of non-amino acid selenocompounds such as SeMSeCys and GGSeCys. Thus, they can accumulate high Se without Se toxicity caused by incorporation of SeMet and SeCys into proteins. In addition, SMT is a key enzyme in producing non-amino selenocompounds in Se hyperaccumulator plants. In the current study, most Se accumulated as SeMet in the seeds and meal and SeMSeCys accumulated rapidly in leaves, being detected only 6 h after application. Therefore, Total Se content, Se speciation analysis of Se compounds and amino acid sequence of SMT enzyme showed that oilseed rape is a non-accumulator of Se.

The Se-enriched plant residues were not able to promote the Se accumulation to the target level. This was considered to be attributable to their slow biodegradation. Thus, they cannot efficiently recycle Se in agroecosystems and reduce the annual need of inorganic fertilizer Se, so inorganic fertilizer Se is still recommended for Se biofortification in Finland. Further studies are needed to unravel the mechanisms underlying the decomposition of Se-enriched residues and their fate in nutrient cycling in agroecosystems.

Autoclaving of soil resulted in higher Se accumulation in the dry matter of plant and seed as well as in a higher Se content in the plants and a higher recovery of fertilizer Se. Autoclaving may promote the cell lysis of microorganisms and the release of nutrients to the soil solution and, thus, to improve plant growth and Se accumulation. Thus, in the big scale soil steam sterilization can be used as a tool for killing the pests such as weeds, fungi, bacteria and viruses in soil with releasing the blocked nutrients into soil solution. The plants that are cultivated in this background are healthy and vigorous. Another benefit from soil steam sterilization method can be reducing the use of chemicals to control the pests, especially in organic farm systems.

Nevertheless, the field study showed that plant species and the conditions of the growing season are important factors affecting the recovery of fertilizer Se. As for leafy crops, forage grasses were more efficient in Se uptake than the grain crops (wheat and oilseed rape). In addition, wheat plants had a higher capacity to accumulate Se in their grains than the oilseed rape, though the total Se uptake was lower. The storage proteins of wheat grains are rich in the S-containing amino acids Met and Cys and thus may provide a large sink for Se accumulation. In 2012, high rainfall drastically reduced the recovery of fertilizer Se. This effect may have occurred due to leaching of selenate along with changes in pH and redox conditions of soil that converted selenate to reduced forms and favored their adsorption onto Al and Fe (oxy)hydroxides in soil.

Our results indicate that the Se biofortification pathway has two main bottlenecks. The first one is the bioavailability of Se for uptake by plants and the second one is the limited capacity of plants to load Se into the seeds. The data on Se remobilization, its uptake and recovery efficiency in oilseed rape plants, were used to outline a schematic model of Se cycling in field agroecosystems. Only a small portion of Se is transferred into the food chain or volatilized into atmosphere. Most of the applied Se remains in

soil or is returned to soil with Se-enriched plant residues. In addition, the Se-enriched plant residues did not efficiently recycle Se in the agroecosystem. Thus, the use of inorganic Se fertilizer is still an indispensable strategy for Se biofortification in Finland. Overall, to improve the recovery of fertilizer Se, further studies on the remobilization of Se and its loading into the seeds are needed. Further studies are also needed on the physiological and molecular mechanisms to overcome the obstacles in the biofortification pathway and to reduce the amount of annual Se fertilizer application.

REFERENCES

- Abdulah R, Miyazaki K, Nakazawa M, Koyama H. (2005). Chemical forms of selenium for cancer prevention. *Journal of Trace Elements in Medicine and Biology* 19(2-3): 141–150.
- Adriano DC. (1986). Trace elements in the terrestrial environment. Springer, New York, NY.
- Ajwa HA, Banuelos GS, Mayland HF. (1998). Selenium uptake by plants from soils amended with inorganic and organic materials. *Journal of Environmental Quality* 27(5): 1218–1227.
- Alemi MH, Goldhamer DA, Nielsen DR. (1988). Selenate transport in steady-state, water-saturated soil columns. *Journal of Environmental Quality* 17(4): 608–613.
- Alfthan G, Eurola M, Ekholm P, Venäläinen ER, Root T, Korkalainen K, Hartikainen H, Salminen P, Hietaniemi V, Aspila P, Aro A. (2015). Effects of nationwide addition of selenium to fertilizers on foods, and animal and human health in Finland: From deficiency to optimal selenium status of the population. *Journal of Trace Elements in Medicine and Biology* 31:142–147.
- Alfthan G, Aspila P, Ekholm P, Eurola M, Hartikainen H, Hero H, Hietaniemi V, Root T, Salminen P, Venäläinen ER, Aro A. (2010). Nationwide Supplementation of Sodium Selenate to Commercial Fertilizers: History and 25-year Results from the Finnish Selenium Monitoring Programme. In: Thompson B, Amoroso L, editors. Combating micronutrient deficiencies: Food- based approaches. Rome: FAO/CAB International. 17: 312–337.
- Avice JC, Etienne P. (2014). Leaf senescence and nitrogen remobilization efficiency in oilseed rape (*Brassica napus* L.). *Journal of Experimental Botany* 65(14): 3813–3824.
- Bahners N. (1987). Selengehalte von Böden und deren Grasaufwuchs in der Bundesrepublik sowie Möglichkeiten der Selenanreicherung durch verschiedene Selen Düngungen. PhD thesis, Friedrich Wilhelms Universität, Bonn, pp.151.
- Banuelos GS, Arroyo I, Pickering IJ, Yang SI, Freeman JL. (2015). Selenium biofortification of broccoli and carrots grown in the soil amended with Se-enriched hyperaccumulator *Stanleya pinnata*. *Food Chemistry* 166: 603–608.
- Banuelos GS, Walse SS, Yang S I, Pickering IJ, Fakra SC, Marcus MA, Freeman JL. (2012). Quantification, localization, and speciation of selenium in seeds of canola and two mustard species compared to seed-meals produced by hydraulic press. *Analytical Chemistry* 84(14): 6024–6030.
- Bentley R, Chasteen TG. (2002). Microbial methylation of metalloids: Arsenic, antimony, and bismuth. *Microbiology and Molecular Biology Reviews* 66(2): 250–271.
- Bianga J, Govasmark E, Szpunar J. (2013). Characterization of selenium incorporation into wheat proteins by two-dimensional gel electrophoresis–laser ablation ICP MS followed by capillary HPLC–ICP MS and electrospray linear trap quadrupole orbitrap MS. *Analytical Chemistry* 85(4): 2037–2043.
- Bisbjerg B, Gissel-Nielsen G. (1969). The uptake of applied selenium by agricultural plants: I. the influence of soil type and plants species. *Plant and Soil* 31: 287–298.
- Bitterli C, Banuelos GS, Schulin R. (2010). Use of transfer factors to characterize uptake of selenium by plants. *Journal of Geochemical Exploration* 107(2): 206–216.
- Bloem E, Haneklaus S, Schung E. (2007). Changes in the sulfur and glucosinolate content of developing pods and seeds of oilseed rape (*Brassica napus* L.) in relation to different cultivars. *Landbauforschung Völkenrode* 57: 297–306.

- Boldrin PF, Faquin V, Ramos SJ, Boldrin KVF, Ávil FW, Guilherme LRG. (2013). Soil and foliar application of selenium in rice biofortification. *Journal of Food Composition and Analysis* 31(2): 238–244.
- Broadley MR, Alcock J, Alford J, Cartwright P, Foot I, Fairweather-Tait SJ, Hart DJ, Hurst R, Knott P, McGrath SP, et al. (2010). Selenium biofortification of high-yielding winter wheat (*Triticum aestivum* L.) by liquid or granular Se fertilization. *Plant and Soil* 332: 5–18.
- Broadley MR, White PJ, Bryson RJ, Meacham MC, Bowen HC, Johnson SE, Hawkesford MJ, McGrath SP, Zhao FJ, Breward N. (2006). Biofortification of UK food crops with selenium. *Proceedings of the Nutrition Society* 65: 169–181.
- Brown TA, Shrift A. (1981). Exclusion of selenium from proteins in selenium-tolerant *Astragalus* species. *Plant Physiology* 67:1951–1953.
- Brown TA, Shrift A. (1982). Selenium-toxicity and tolerance in higher plants. *Biological Reviews of the Cambridge Philosophical Society* 57: 59–84.
- Burnell JN. (1981). Selenium Metabolism in *Neptunia amplexicaulis*. *Plant Physiology* 67: 316–324.
- Cao MJ, Wang Z, Wirtz M, Hell R, Oliver DJ, Xiang CB. (2013). SULTR31 is a chloroplast-localized sulphate transporter in *Arabidopsis thaliana*. *The Plant Journal* 73: 607–616.
- Cartes P, Jara AA, Pinilla L, Rosas A, Mora ML. (2010). Selenium improves the antioxidant ability against aluminum-induced oxidative stress in ryegrass roots. *Annals of Applied Biology* 156(2): 297–307.
- Chen YW, Thi-Truong HY, Belzile N. (2009). Abiotic formation of elemental selenium and role of iron oxide surfaces. *Chemosphere* 74(8): 1079–1084.
- Chu J, Yao X, Zhang Z. (2010). Responses of wheat seedlings to exogenous selenium supply under cold stress. *Biological Trace Element Research* 136(3): 355–363.
- Cubadda F, Aureli F, Ciardullo S, D'Amato M, Raggi A, Raghunath Acharya Reddy RAV, Prakash NT. (2010). Changes in selenium speciation associated with increasing tissue concentrations of selenium in wheat grain. *Journal of Agricultural and Food Chemistry* 58(4): 2295–2301.
- Curtin D, Hanson R, Lindley TN, Butler RC. (2006). Selenium concentration in wheat (*Triticum aestivum*) grain as influenced by method, rate, and timing of sodium selenate application. *New Zealand Journal of Crop and Horticultural Science* 34: 329–339.
- De Souza MP, Chu D, May Z, Zayed AM, Ruzin SE, Schichnes D, Terry N. (1999). Rhizosphere bacteria enhance selenium accumulation and volatilization by Indian mustard. *Plant Physiology* 199: 565–573.
- Dhillon SK, Dhillon KS. (2000). Selenium adsorption in soils as influenced by different anions. *Journal of Plant Nutrition and Soil Science* 163(6): 577–582.
- Di Gregorio S, Lampis S, Vallini G. (2005). Selenite precipitation by a rhizospheric strain of *Stenotrophomonas* sp. isolated from the root system of *Astragalus bisulcatus*: a biotechnological perspective. *Environment International* 31(2): 233–241.
- Djanaguiraman M, Durga DD, Shanker AK, Sheeba JA, Bangarusamy U. (2005). Selenium, an antioxidative protectant in soybean during senescence. *Plant and Soil* 272: 77–86.

- Djanaguiraman M, Prasad PVV, Seppänen M. (2010). Selenium protects sorghum leaves from oxidative damage under high temperature stress by enhancing antioxidant defense system. *Plant Physiology and Biochemistry* 48: 999–1007.
- Dumont E, De Pauw L, Vanhaecke F, Cornelis R. (2006). Speciation of Se in *Bertholletia excelsa* (Brazil nut): a hard nut to crack? *Food Chemistry* 95(4): 684–692.
- Dungan RS, Frankenberger WT. (1999). Microbial transformations of selenium and the bioremediation of seleniferous environments. *Bioremediation Journal* 3(3):171–188.
- Dunn PH, DeBano LF, Eberlein GE. (1979). Effects of burning on chaparral soils: II Soil microbes and nitrogen mineralization. *Soil Science Society of America Journal* 43(3): 509–514.
- Durán P, Acuña JJ, Jorquera MA, Azcón R, Borie F, Cornejo P, Mora ML. (2013). Enhanced selenium content in wheat grain by co-inoculation of selenobacteria and arbuscular mycorrhizal fungi: A preliminary study as a potential Se biofortification strategy. *Journal of Cereal Science* 57(3): 275–280.
- Eich-Greatorex S, Sogn TA, Øgaard AF, Aasen I. (2007). Plant Availability of inorganic and organic selenium fertilizer as influenced by soil organic matter content and pH. *Nutrient Cycling in Agroecosystems* 79(3): 221–231.
- El Mehdawi AF, Paschke MW, Pilon-Smits EAH. (2015). *Symphyotrichum ericoides* populations from seleniferous and nonseleniferous soil display striking variation in selenium accumulation. *New Phytologist* 206(1): 231–242.
- El Mehdawi AF, Pilon-Smits EAH. (2012). Ecological aspects of plant selenium hyperaccumulation. *Plant Biology* 14(1): 1–10.
- Elrashidi MA, Adriano DC, Workman AM, Lindsay WL. (1987). Chemical equilibria of selenium in soils: a theoretical development. *Soil Science* 144: 141–152.
- Elstner EF. (1991). Mechanisms of oxygen activation in different compartments of plant cells. In EJ Pell and KL Steffen (eds.) *Active Oxygen/Oxidative Stress and Plant metabolism*. American Society of Plant Physiologists, Rockville, MD, USA, pp. 13–25.
- Etienne P, Diquelou S, Prudent M, Salon C, Maillard A, Ourry A. (2018). Macro and Micronutrient Storage in Plants and Their Remobilization When Facing Scarcity: The Case of Drought. *Agriculture* 8: 14.
- Eurola M, Alftan G, Ekholm P, Root T, Suoniitti T, Venalainen ER, Ylivainio K. (2011). Report of the selenium working group 2011. MTT Raportti 35. (In Finnish with English abstract).
- Eurola M, Ekholm P, Ylinen M, Kovistoinen P, Varo P. (1990). Effects of selenium fertilization on the selenium content of cereals grains, flour, and bread production in Finland. *Cereal Chemistry* 67: 334–337.
- Fairweather-Tait SJ, Bao Y, Broadley MR, Collings R, Ford D, Hesketh JR, Hurst R. (2011). Selenium in human health and disease. *Antioxidants and Redox Signaling* 14(7): 1337–1383.
- Feist LJ, Parker DR. (2001). Ecotypic variation in selenium accumulation among populations of *Stanleya pinnata*. *New Phytologist* 149(1): 61–69.
- Feng R, Wei C, Tud S. (2013). The roles of selenium in protecting plants against abiotic stresses. *Environmental and Experimental Botany* 87: 58–68.
- Fordyce FM. (2007). Selenium geochemistry and health. *Ambio: A Journal of the Human Environment* 36: 94–97.
- Fordyce FM. (2013). Essentials of medical geology: revised edition, in: O. Selinus, B. Alloway, J.A. Centeno, R. Finkelman, R. Fuge, U. Lindh, P.L. Smedley (Eds.), *Selenium*

- Deficiency and Toxicity in the Environment, Springer, Dordrecht, Netherlands, pp. 375–416.
- Frankenberger WT, Karlson U. (1994). Soil management factors affecting volatilization of selenium from dewatered sediments. *Geomicrobiology Journal*. 12(4): 265–278.
- Freeman JL, Lindblom SD, Quinn CF, Fakra S, Marcus MA, Pilon-Smits EAH. (2007). Selenium accumulation protects plants from herbivory by Orthoptera via toxicity and deterrence. *New Phytologist* 175(3): 490–500.
- Galeas ML, Zhang LH, Freeman JL, Wegner M, Pilon-Smits EAH. (2007). Seasonal fluctuations of selenium and sulfur accumulation in selenium hyperaccumulators and related non-accumulators. *New Phytologist* 173(3): 517–525.
- Gissel-Nielsen G, Gupta UC, Lamand M, Westermarck T. (1984). Selenium in soils and plants and its importance in livestock and human nutrition. *Advances in Agronomy* 37: 397–460.
- González-Morales S, Pérez-Labrada F, García-Enciso EL, Leija-Martínez P, Medrano-Macías J, Dávila-Rangel IE, Juárez-Maldonado A, Rivas-Martínez EN, Benavides-Mendoza A. (2017). Selenium and sulfur to produce *Allium* functional crops. *Molecules* 22(4): 558.
- Govasmarm E, Salbu B. (2011). Translocation and re-translocation of selenium taken up from nutrient solution during vegetative growth in spring wheat. *Journal of the Science of Food and Agriculture* 91(8): 1367–1372.
- Gupta UC, Gupta SC. (2000). Selenium in soils and crops, its deficiencies in livestock and humans: Implications for management. *Communications in Soil Science and Plant Analysis* 31(11-14):1791–1807.
- Hajiboland R, Amjad L. (2007). Does antioxidant capacity of leaves play a role in growth response to selenium at different sulfur nutritional status? *Plant, Soil and Environment* 53: 207–215.
- Hajiboland R, Sadeghzadeh N, Ebrahimi N, Sadeghzadeh B, Mohammadi SA. (2015). Influence of selenium in drought-stressed wheat plants under greenhouse and field conditions. *Acta Agriculturae Slovenica* 105(2): 175–191.
- Hartikainen H, Ekholm P, Piironen V, Xue T, Koivu T, Yli-Halla M. (1997). Quality of the ryegrass and lettuce yields as affected by selenium fertilization. *Agricultural and Food Science* 6: 381–387.
- Hartikainen H, Xue TL, Piironen V. (2000). Selenium as an anti-oxidant and pro-oxidant in ryegrass. *Plant and Soil* 255(1-2): 193–200.
- Hartikainen H, Xue TL. (1999). The promotive effect of selenium on plant growth as triggered by UV irradiation. *Journal of Environmental Quality* 28(4): 1372–1375.
- 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(4): 309–318.
- Hasanuzzaman M, Hossain MA, Fujita M. (2012). Exogenous selenium pretreatment protects rapeseed seedling from cadmium- induced oxidative stress by upregulating antioxidant defense and methylglyoxal detoxification systems. *Biological Trace Element Research* 149(2): 248– 261.
- Haug A, Graham RD, Christophersen OA, Graham HL. (2007). How to use the world's scarce selenium resources efficiently to increase the selenium concentration in food. *Microbial Ecology in Health and Disease* 19: 209–228.
- Hawkesford MJ, Zhao FJ. (2007). Strategies for increasing the selenium content of wheat. *Journal of Cereal Science* 46(3): 282–292.

- Hawrylak-Nowak B. (2009). Beneficial effects of exogenous selenium in cucumber seedlings subjected to salt stress. *Biological Trace Element Research* 132(1-3): 259–269.
- Hawrylak-Nowak B. (2013). Comparative effects of selenite and selenate on growth and selenium accumulation in lettuce plants under hydroponic conditions. *Plant Growth and Regulation* 70(2): 149–157.
- Hayes KF, Lawrence Roe A, Brown GE, Hodgson KO, Leckie JO, Parks GA. (1987). In situ X-ray absorption study of surface complexes: Selenium oxyanions on α -FeOOH. *Science* 238(4828): 783–786.
- Haygarth PM, Cooke AI, Jones KC, Harrison AF, Johnston AE. (1993). Long-term change in the biogeochemical cycling of atmospheric selenium: Deposition to plants and soil. *Journal of Geophysical Research Atmospheres* 98(9): 16769–16776.
- Haygarth PM, Fowler D, Stvirup S, Davison BM, Jones K.C. (1994). Determination of gaseous and particulate selenium over a rural grassland in the U.K. *Atmospheric Environment* 28(22): 3655–3663.
- Hoffmann JE, King MG. (1997). Selenium and selenium compounds in Kirk- Othmer encyclopedia of chemical technology (4th ed): New York, Wiley and Sons 21: 686–719.
- Huang TS, Shyu YC, Chen HY, Lin LM, Lo CY, Yuan SS, et al. (2013). Effect of parental selenium supplementation in critically III patients: A systematic review and meta-analysis. *PLoS ONE* 8(1): e54431.
- Issa AA, Adam MS. (1999). Influence of selenium on toxicity of some heavy metals in the green alga *Scenedesmus obliquus*. *Folia Microbiologia* 44(4): 406–410.
- Johnsson L. (1991). Selenium uptake by plants as a function of soil type, organic matter content and pH. *Plant and Soil* 133: 57–64.
- Keskinen R, Rätty M, Yli-Halla M. (2011). Selenium fractions in selenate-fertilized field soils of Finland. *Nutrient Cycling in Agroecosystems* 91(1): 17–29.
- Keskinen R, Yli-Halla M, Hartikainen H. (2013). Retention and uptake by plants of added selenium in peat soils. *Communications in Soil Science and Plant Analysis* 44(22): 3465–3482.
- Keskinen R. (2012). Selenium Fertilization: Plant Uptake and Residuals in Soil. Doctoral thesis. MTT Agrifood Research Finland, ISSN 1798-1840.
- Kikkert J, Berkelaar E. (2013). Plant uptake and translocation of inorganic and organic forms of selenium. *Archives of Environmental Contamination and Toxicology* 65: 458–465.
- Koljonen T. (1974). Selenium in certain Finnish sediments. *Bulletin of the Geological Society of Finland* 46: 15–21.
- Koljonen T. (1975). The behavior of selenium in Finnish soils. *Annales Agriculture Fenniae* 14: 240–247.
- König J, Muthuramalingam M, Dietz KJ. (2012). Mechanisms and dynamics in the thiol/disulfide redox regulatory network: transmitters, sensors and targets, *Current Opinion in Plant Biology* 15(3): 261–268.
- Kubachka KM, Meija J, LeDuc DL, Terry N, Caruso JA. (2007). Selenium volatiles as proxy to the metabolic pathways of selenium in genetically modified *Brassica juncea*. *Environmental Science and Technology* 41(6): 1863–1869.
- Kutiel P, Shaviv A. (1989). Effect of simulated forest fire on the availability of N and P in Mediterranean soils. *Plant and Soil* 120: 57–63.

- Kutman UB, Yildiz B, Cakmak I (2011) Effect of nitrogen on uptake, remobilization and partitioning of zinc and iron throughout the development of durum wheat. *Plant and Soil* 342: 149–164.
- LeDuc DL, AbdelSamie M, Montes-Bayon M, Wu CP, Reisinger SJ, Terry N. (2006). Overexpressing both ATP sulfurylase and selenocysteine methyltransferase enhances selenium phytoremediation traits in Indian mustard. *Environmental Pollution* 144(1): 70–76.
- Lee J, Masters DG, White CL, Grace ND, Judson GJ. (1999). Current issues in trace element nutrition of grazing livestock in Australia and New Zealand. *Australian Journal of Agricultural Research* 50: 1341–1364.
- Lee S, Woodard HJ, Doolittle JJ. (2011). Selenium uptake response among selected wheat (*Triticum aestivum*) varieties and relationship with soil selenium fractions. *Soil Science and Plant Nutrition* 57(6): 823–832.
- Li S, Schonhof I, Krumbein A, Li L, Stützel H, Schreiner M. (2007). Glucosinolate concentration in turnip (*Brassica rapa* ssp. *rapifera* L.) roots as affected by nitrogen and sulfur supply. *Journal of Agricultural and Food Chemistry* 55(21): 8452–8457.
- Lin ZQ, Schemenaur RS, Cervinka V, Zayed A, Lee A, Terry N. (2000). Selenium volatilization from a soil-plant system for the remediation of contaminated water and soil in the San Joaquin Valley. *Journal of Environmental Quality* 29(4): 1048–1056.
- Lindblom SD, Valdez-Barillas JR, Fakra SC, Marcus MA, Wangeline AL, Pilon-Smits EAH. (2013). Influence of microbial associations on selenium localization and speciation in roots of *Astragalus* and *Stanleya* hyperaccumulators. *Environmental and Experimental Botany* 88: 33–42.
- Livak KJ, Schmittgen TD. (2001). Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the $2^{-\Delta\Delta CT}$ Method. *Methods* 25(4): 402–408.
- Lokeshappa B, Shivpuri K, Tripathi V, Dikshit AK. (2012). Assessment of toxic metals in agricultural produce. *Food and Public Health* 2(1): 24–29.
- Lyons GH, Gene Y, Soole K, Stangoulis JCR, Liu F, Graham RD. (2009). Selenium increases seed production in *Brassica*. *Plant and Soil* 318(1-2): 73–80.
- Lyons GH, Lewis J, Lorimer MF, Holloway RE, Brace DM, Stangoulis JCR, Graham RD. (2004). High-selenium wheat: agronomic biofortification strategies to improve human nutrition. *Journal of Food Agriculture and Environment* 2(1): 171–178.
- Lyons GH. (2010). Selenium in cereals: Improving the efficiency of agronomic biofortification in the UK. *Plant and Soil* 332: 1–4.
- Maillard A, Diquélou S, Billard V, Lâiné P, Garnica M, Prudent M, Garcia-Mina JM, Yvin JC, Ourry A. (2015). Leaf mineral nutrient remobilization during leaf senescence and modulation by nutrient deficiency. *Frontier in Plant Science* 13.
- Malik J, Goela S, Kaura N, Sharma S, Singh I, Nayyar H. (2011). Selenium antagonises the toxic effects of arsenic on mungbean (*Phaseolus aureus* Roxb.) plants by restricting its uptake and enhancing the antioxidative and detoxification mechanisms. *Environmental and Experimental Botany* 77: 242–248.
- Mehdi Y, Hornick JL, Istasse L, Dufrasne I. (2013). Selenium in the environment, metabolism and involvement in body functions. *Molecules* 18(3): 3292–3311.
- Miller DD, Welch RS. (2013). Food system strategies for preventing micronutrient malnutrition. *Food Policy* 42: 115–128.
- Mittler R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science* 7(9): 405–410.

- Moxon AL, Rhian M. (1943). Selenium poisoning. *Physiological Reviews* 23: 305–337.
- Nawaz F, Ahmad R, Ashraf M, Waraich E, Khan S. (2015). Effect of selenium foliar spray on physiological and biochemical processes and chemical constituents of wheat under drought stress. *Ecotoxicology and Environmental Safety* 113: 191–200.
- Oksanen HE, Sanholm M. (1970). The selenium content of Finnish forage crops. *Journal of the Scientific Agricultural Society Finland* 42: 250–253.
- Oldfield JE, Muth OH, Schubert JR. (1960). Selenium and vit. E as related to growth and white muscle disease in Lambs. *Proceedings of the Society for Experimental Biology and Medicine* 103(4): 799–800.
- Oldfield JE. (2002). *Selenium World Atlas: Updated Edition; Selenium-Tellurium Dev. Assoc.: Grimbergen, Belgium.*
- Owusu-Skyere A, Kontturi J, Hajiboland R, Rahmat S, Aliasghar zad N, Hartikainen H, Seppänen MM. (2013). Influence of selenium (Se) on carbohydrate metabolism, nodulation and growth in alfalfa (*Medicago sativa* L.). *Plant and Soil* 373: 541–552.
- Pezzarossa B, Remorini D, Gentile ML, Massay R. (2012). Effects of foliar and fruit addition of sodium selenate on selenium accumulation and fruit quality. *Journal of the Science of Food and Agriculture*. 92(4): 781–786.
- Pilon M, Owen JD, Garifullina GF, Kurihara T, Mihara H, Esaki N, Pilon-Smits EHA, (2003). Enhanced selenium tolerance and accumulation in transgenic *Arabidopsis* expressing a mouse selenocysteine lyase. *Plant Physiology* 132(1): 1250–1257.
- Pilon-Smits EAH, Quinn CF, Tapken W, Malagoli M, Schiavon M. (2009). Physiological functions of beneficial elements. *Current Opinion in Plant Biology* 12: 267–274.
- Pilon-Smits EAH, Quinn CF. (2010). Selenium metabolism in plants. In *Cell Biology of Metals and Nutrients*; Hell, R., Mendel, R.-R., Eds.; Springer: Berlin/Heidelberg, Germany 17: 225–241.
- Poblaciones MJ, Rodrigo S, Santamaría O, Chen Y, McGrath SP. (2014). Agronomic selenium biofortification in *Triticum durum* under Mediterranean conditions: From grain to cooked pasta. *Food Chemistry* 146: 378–384.
- Pommerrenig B, Diehn TA, Bienert GP. (2015). Metalloid-porins: essentiality of Nodulin 26-like intrinsic proteins in metalloid transport. *Plant Science* 238: 212–227.
- Qing X, Zhao X, Hu C, Wang P, Zhang Y, Zhang X, Wang P, Shi H, Jia F, Qu C. (2015). Selenium alleviates chromium toxicity by preventing oxidative stress in cabbage (*Brassica campestris* L. ssp. *Pekinensis*) leaves. *Ecotoxicology and Environmental Safety Journal* 114:179–189.
- Ramkissoo C, Degryse F, da Silva RC, Baird R, Young SD, Bailey EH, McLaughlin MJ. (2019). Improving the efficacy of selenium fertilizers for wheat biofortification. *Scientific Reports* 9: 19520.
- Renkema H, Koopmans A, Kersbergen L, Kikkert J, Hale B, Berkelaar E. (2012). the effect of transpiration on selenium uptake and mobility in durum wheat and spring canola. *Plant and Soil* 354: 239–250.
- Rimmer DL, Shiel RS, Syers JK, Wilkinson M. (1990). Effects of Soil Application of Selenium on Pasture Composition. *Journal of the Science of Food and Agriculture* 51(3): 407–410.
- Roman M, Jitaru P, Barbante C. (2014). Selenium biochemistry and its role for human health. *Metallomics* 6(1): 25–54.

- Ros GH, van Rotterdam AMD, Bussink DW, Bindraban PS. (2016). Selenium fertilization strategies for bio-fortification of food: an agro-ecosystem approach. *Plant and Soil* 404:99–112.
- Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. (1973). Selenium: Biochemical role as a component of glutathione peroxidase. *Science* 179(4073): 588–590.
- Saha UK, Liu C, Kozak LM, Huang PM. (2004). Kinetics of selenite adsorption on hydroxyaluminium and hydroxyaluminosilicate-montmorillonite complexes. *Soil Science Society of America Journal* 68: 1197–1209.
- Schwarz K, Foltz CM. (1957). Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. *Journal of the American Chemical Society* 79(12): 3292–3293.
- Seby F, Potin-Gautier M, Giffaut E, Borge G, Donard OFX. (2001). A critical review of thermodynamic data for selenium species at 25 °C. *Chemical Geology* 171: 173–194.
- Seppänen MM, Kontturi J, Lopez Heras I, Madrid Y, Camara C, Hartikainen H. (2010). Agronomic biofortification of *Brassica* with selenium-enrichment of SeMet and its identification in *Brassica* seeds and meal. *Plant and Soil* 337: 273–283.
- Serrasolses I, Romanya J, Khanna PK. (2008). Effects of heating and autoclaving on sorption and desorption of phosphorus in some forest soils. *Biology and Fertility of Soils* 44:1063–1072.
- Shand CA, Eriksson J, Dahlin AS, Lumsdon DG. (2012). Selenium concentrations in national inventory soils from Scotland and Sweden and their relationship with geochemical factors. *Journal of Geochemical Exploration* 121: 4–14.
- Shrift A, Ulrich JM. (1969). Transport of selenate and selenite into *Astragalus* roots. *Plant Physiology* 44 (1969): 893–896.
- Sieprawska A, Kornaś A, Filek M. (2015). Involvement of selenium in protective mechanisms of plants under environmental stress conditions – review. *Acta Biologica Cracoviensia s. Botanica* 57: 9–20.
- Sillanpää M, Jansson H. (1992). Status of cadmium, lead, cobalt and selenium in soils and plants of thirty countries. *FAO Soil Bulletin* 65: 195.
- Singh M, Singh N, Banhdari DK. (1980). Interaction of selenium and sulfur on the growth and chemical composition of ray. *Soil Science* 129: 238–244.
- Sippola J. (1979). Selenium content of soils and timothy (*Phleum pretense* L.) in Finland. *Annales Agriculturae Fenniae* 18: 182–187.
- Sors TG, Ellis DR, Salt DE. (2005). Selenium uptake, translocation, assimilation and metabolic fate in plants. *Photosynthesis Research* 86: 373–389.
- Spadoni M, Voltaggio M, Carcea 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. *Science of the Total Environment* 376:160–177.
- Srikanth-Lavu RV, Schepper VD, Steppe K, Majeti PNV, Tack F, Laing GD. (2013). Use of selenium fertilization for production of Se- enriched Kenaf (*Hibiscus cannabinus*): Effect on Se concentration and plant productivity. *Journal of Plant Nutrition and Soil Science* 176 (4): 634–639.
- Stadlober M, Sager M, Irgoic KJ. (2001). Effects of selenite supplemented fertilization on the selenium level of cereals-identification and quantification of selenium compounds by HPLC-ICP-MS. *Food Chemistry* 73: 357–366.

- Stavridou E, Thorup-Kristensen K, Young S. (2011). Assessment of selenium mineralization and availability from catch crops. *Soil Use and Management* 27 (3): 305–311.
- Stavridou E, Young SD, Thorup-Kristensen K. (2012). The effect of catch crop species on selenium availability for succeeding crops. *Plant and Soil* 351:149–160.
- Stephen RC, Saville DJ, Watkinson JH. (1989). The effects of sodium selenate applications on growth and selenium concentration in wheat. *New Zealand Journal of Crop and Horticultural Science* 17: 229–237.
- Stolz JF, Basu P, Santini JM, Oremland RS. (2006). Arsenic and selenium in microbial metabolism. *Annual Review of Microbiology* 60(1): 107–130.
- Stroud JL, Broadley MR, Foot I, Fairweather-Tait SJ, Hart DJ, Hurst R, Knott P, Mowat H, Norman K, Scott P, Tucker M, White PJ, McGrath SP, Zhao FJ. (2010). Soil factors affecting selenium concentration in wheat grain and the fate and speciation of Se fertilizers applied to soil. *Plant and Soil* 332: 19–30.
- Sun QA, Wu Y, Zappacosta F, Jeang KT, Lee BJ, Hatfield DL, Gladyshev VN. (1999). Redox regulation of cell signaling by selenocysteine in mammalian thioredoxin reductases. *Journal of Biological and Chemistry* 274(35): 24522–24530.
- Tan J, Zhang D, Hou S, Zhu W, Li R. (1987). Selenium ecological chemogeography and endemic Keshan disease and Kashin-Beck disease in China. In: Combs GF, Spallholz JE, Levander OA, Oldfield JE. Editors. *Selenium in Biology and Medicine*. Third International Symposium 1984 Beijing. New York: AVI Book Published by Van Nostrand Reinhold Company 839–876.
- Tegeder M. (2012). Transporters for amino acids in plant cells: some functions and many unknowns. *Current Opinion in Plant Biology* 15: 315–321.
- Terry N, Zayed AM, de Souza MP, Tarun AS. (2000). Selenium in higher plants. *Annual Reviews of Plant Physiology and Plant Molecular Biology* 51: 401–432.
- Trinsourtrot I, Recous S, Mary B, Nicolardot, B. (2000). C and N fluxes of decomposing ¹³C and ¹⁵N *Brassica napus* L. effects of residue composition and N content. *Soil Biology and Biochemistry* 32: 1717–1730.
- Turakainen M, Hartikainen H, Ekholm P, Seppänen MM. (2006). Distribution of selenium in different biochemical fractions and raw darkening degree of potato (*Solanum tuberosum*) tubers supplemented with selenate. *Journal of Agricultural and Food Chemistry* 54: 8617–8622.
- Turakainen M, Hartikainen H, Seppänen MM. (2004). Effects of selenium treatments on potato (*Solanum tuberosum* L.) growth and concentrations of soluble sugars and starch. *Journal of Agricultural and Food Chemistry* 52: 5378–5382.
- Turner TR, James EK, Poole PS. (2013). The plant microbiome. *Genome Biology* 14: 209.
- Uauay C, Brevis JC, Dubcovsky J. (2006). The high grain protein content gene Gpc-B1 accelerates senescence and has pleiotropic effects on protein content in wheat. *Journal of Experimental Botany* 57(11): 2785–2794.
- Van Dorst SH, Peterson PJ. (1984). Selenium speciation in the soil solution and its relevance to plant uptake. *Journal of the Science of Food and Agriculture* 35(6): 601–605.
- 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: 965–972.
- Varo P, Koivistoinen P. (1980). Mineral element composition in Finnish foods XII. General discussion and nutritional evaluation. In: Koivistoinen P. (Ed). *Mineral*

- element composition of Finnish foods: N, K, Ca, Mg, P, S, Fe, Cu, Mn, Zn, Mo, Co, Ni, Cr, F, Se, Si, Rb, Al, B, Br, Hg, As, Cd, Pb and Ash. *Acta Agriculturae Scandinavica* 22: 165–171.
- Wang S, Liang D, Wang D, Wei W, Fu D, Lin Z. (2012). Selenium fractionation and speciation in agriculture soils and accumulation in corn (*Zea mays* L.) under field conditions in Shaanxi Province, China. *Science of the Total Environment*. 15 (427–428): 159–164.
- Wangeline AL, Valdez JR, Lindblom SD, Bowling KL, Reeves FB, Pilon-Smits EHA. (2011). Characterization of rhizosphere fungi from selenium hyperaccumulator and nonhyperaccumulator plants along the eastern Rocky Mountain Front Range. *American Journal of Botany*. 98(7): 1139–1147.
- Waters B, Sankaran RP. (2011). Moving micronutrients from the soil to the seeds: Genes and physiological processes from a biofortification perspective. *Plant Science* 180(4): 562–574.
- Watkinson JH. (1983). Prevention of selenium deficiency in grazing animals by annual topdressing of pasture with sodium selenate. *New Zealand Veterinary Journal* 31: 78–85.
- Wenzel WW. (2009). Rhizosphere processes and management in plant-assisted bioremediation (phytoremediation) of soils. *Plant and Soil* 321: 385–408.
- White PJ, Bowen HC, Parmaguru P, Fritz M, Spracklen WP, Spiby RE, Meacham MC, Mead A, Harriman M, Trueman LJ, Smith BM, Thomas B, Broadley MR. (2004). Interactions between selenium and sulphur nutrition in *Arabidopsis thaliana*. *Journal of Experimental Botany* 55(404): 1927–1937.
- White PJ. (2016). Selenium accumulation by plants. *Annals of Botany* 117(2): 217–235.
- White PJ. (2018). Selenium metabolism in plants. *Biochimica et Biophysica Acta General Subjects* 1862(11): 2333–2342.
- Wichtel JJ. (1998). A review of selenium deficiency in grazing ruminants. Part 1: new roles for selenium in ruminant metabolism. *New Zealand Veterinary Journal* 46: 47–52.
- Winkel LHE, Vriens B, Jones GD, Schneider LS, Pilon-Smits E, Banuelos GS. (2015). Selenium cycling across soil–plant–atmosphere interfaces: a critical review. *Nutrients* 7: 4199–4239.
- Xue T, Hartikainen H, Piironen V. (2001). Antioxidative and growth-promoting effect of selenium on senescing lettuce. *Plant and Soil* 237: 55–61.
- Xue TL, Hartikainen H. (2000). Association of antioxidative enzymes with the synergistic effect of selenium and UV irradiation in enhancing plant growth. *Agricultural and Food Science* 9: 177–186.
- Ylärinta T. (1983). Selenium in Finnish agricultural soils. *Annales Agriculturae Fenniae* 22: 122–136.
- Ylärinta T. (1985). Increasing Selenium Content of Cereals and Grass Crops in Finland. Finland: University of Helsinki, 1985, ISBN 951- 729- 269- 4.
- Ylärinta T. (1990). The selenium content of some agricultural crops and soils before and after the addition of selenium to fertilizers in Finland. *Annales Agriculturae Fenniae* 29: 131–139.
- Zayed A, Lytle CM, Terry N. (1998). Accumulation and volatilization of different chemical species of selenium by plants. *Planta* 206: 284–292.

- Zayed AM, Terry N. (1994). Selenium volatilization in roots and shoots: effect of shoot removal and sulfate level. *Journal of Plant Physiology* 143: 8–14.
- Zhang L, Hu B, Li W, Che R, Deng K, Li H, Yu F, Ling H, Li Y, Chu C. (2014). OsPT2, a phosphate transporter, is involved in the active uptake of selenite in rice. *New Phytologist* 201: 1183–1191.
- Zhang Y, Pan G, Chen J, Hu Q. (2003). Uptake and transport of selenite and selenate by soybean seedlings of two genotypes. *Plant and Soil* 253(2): 437–443.
- Zhao XQ, Mitani N, Yamaji N, Shen RF, Ma JF. (2010). Involvement of silicon influx transporter osnip2;1 in selenite uptake in rice. *Plant Physiology* 153: 1871–1877.
- Zhu YG, Pilon-Smits EA, Zhao FJ, Williams PN, Meharg AA. (2009). Selenium in higher plants: Understanding mechanisms for biofortification and phytoremediation. *Trends in Plant Science* 14: 436–447.