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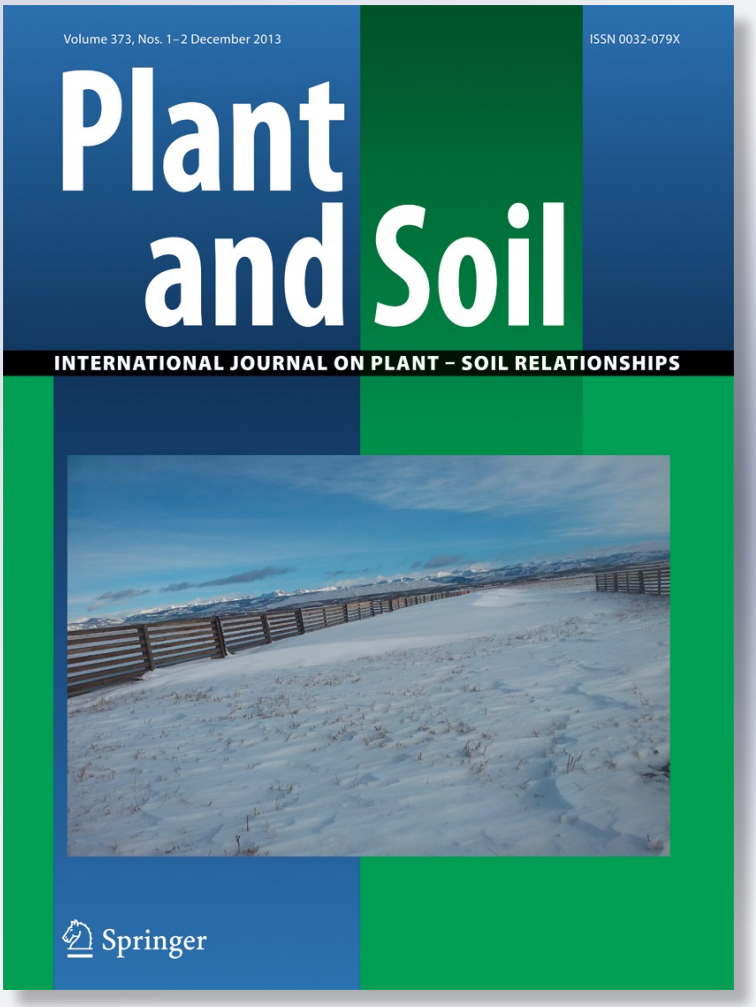

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REGULAR ARTICLE

Influence of selenium (Se) on carbohydrate metabolism, nodulation and growth in alfalfa (*Medicago sativa* L.)

Anthony Owusu-Sekyere · Juha Kontturi · Roghieh Hajiboland · Somaieh Rahmat · Naser Aliasgharzad · Helinä Hartikainen · Mervi M. Seppänen

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Abstract
Background and Aims Selenium (Se) is an essential micronutrient for humans and animals, but its role in plants remains unclear. Selenium enters the food chain via crops, and thus, plants constitute an essential source of Se in human nutrition. As a N_2-fixing plant of high nutritive value, alfalfa (*Medicago sativa* L.) is an important forage legume for sustainable agriculture. This study investigated the effects of Se on carbohydrate metabolism, nodulation and growth in alfalfa. In addition, the impact of Se on fructose 1,6-bisphosphatase (F1,6-BPase), a key enzyme in carbohydrate metabolism, as well as on nitrogenase activity in N-metabolism was examined.

Methods Alfalfa was grown either in perlite or nutrient solution at different Se (0, 1, 5, 10 and 15 μmol L^{-1} Na_2SeO_4) and N (2 and 10 mmol L^{-1}) concentrations. Plants in perlite were inoculated with *Sinorhizobium meliloti* and used for studies on nodulation, growth and nitrogenase activity. Plants grown in nutrient solution were used for studies on carbohydrate metabolism.
Results Selenium applications (5 and 15 μmol L^{-1}) increased soluble sugars (SS) in the leaves, on average, by 44 % in both adequate-N and low-N-plants respectively. At the low-N level, Se (10 and 15 μmol L^{-1}) increased SS in the stems and roots, on average, by 29 % and 45 % respectively. In adequate-N-plants, Se increased SS in the stems, on average, by 46 % but had no effect in the roots. Selenium (10 and 15 μmol L^{-1}) enhanced starch accumulation in the leaves about 55 % in low-N-plants. At the adequate-N level, Se (15 μmol L^{-1}) increased starch accumulation about 36 %. However, the starch concentrations in the roots were inconsistent. Selenium also increased F1,6-BPase activity in the upper leaflets. In addition, in low-N-plants, the low Se (1 μmol L^{-1} and 5 μmol L^{-1}) applications increased nodule number (NN) about 40 % and 62 % respectively, but NN decreased with plant growth. In symbiotic plants, Se did not significantly affect nodule fresh weight (NFW), nitrogenase activity and N concentrations. Selenium also had a slightly negative effect on dry matter accumulation in shoots and roots of alfalfa.
Conclusions The results indicate that, Se up-regulates carbohydrate metabolism via altered redox potential which may have some stimulatory effects on nodulation. These effects were, however,

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dependent on the Se concentration and the developmental stage of the plant. More detailed studies are needed to fully understand the role of Se in N_2 fixation.

Keywords *Medicago sativa* · Selenium · Nitrogen fixation · Nitrogenase · Soluble sugars · Starch · Fructose 1,6-bisphosphatase · Photosynthesis

Introduction

Concern for excessive use and increasing prices of chemical fertilizers increases the economic and ecological importance of biological N_2-fixation (Stinner and Blair 1990). This multi-step process, catalysed by the nitrogenase enzyme, is peculiar to legumes and some prokaryotes and serves as a renewable source of N to plants. Fixed N_2 in the form of ammonia is incorporated into amino acids and other N-containing compounds. Alfalfa is the most important forage legume in temperate countries, including the Mediterranean sub-region (Michaud et al. 1988; references therein). Owing to its high nutritive quality, alfalfa has become a key feed for ruminants, with an estimated global production of over four hundred million tonnes (USDA 2007). It forms a very essential symbiosis with the soil bacterium *Sinorhizobium meliloti* in nodule formation. This involves the expression of specific plant-bacteria genes (Nap and Bisseling 1990). Specific flavonoids secreted by the roots interact with rhizobia signalling molecule lipochitooligosaccharides (Nod factors) (Fisher and Long 1992). The nodule formation and development thus, function as a N source, and this is also closely linked to carbon metabolism in legumes (Hawkers 1985; Joshi et al. 1993).

At trace concentrations, Se is an essential micronutrient for humans and animals, but at higher concentrations (> 400 μg Se d^{-1}) it can be harmful (Terry et al. 2000; references therein). Selenium is a chemical analogue of sulphur (S), and as selenate, it has the same metabolic pathway via sulphate transporters and enzymes (Terry et al. 2000; Sors et al. 2005). Selenium mainly enters the food chain through the diet, and its levels in food depend on the concentration in the soil, plant uptake and plant accumulation capacity (Terry et al. 2000; Hartikainen 2005). In several countries where dietary Se intake is low or deficient, some Se deficiency disorders have been reported in humans and livestock (Coppinger and Diamond 2001).

Although evidence of the beneficial effects of Se of growth in higher plants is increasing, no consensus has been reached on its classification as a plant micronutrient (Hartikainen et al. 2000; Terry et al. 2000; Pilon-Smits et al. 2009; White and Broadley 2009). Hartikainen et al. (2000) reported that at low concentrations, Se exerts antioxidative effects such as enhanced glutathione peroxidase activity and inhibition of lipid peroxidation. Selenium also increases the tolerance of plants to abiotic stresses such as salinity (Hawrylak-Nowak 2009), ultraviolet (UV) radiation (Hartikainen and Xue 1999) and drought (Yao et al. 2009). In addition, at low concentrations (0.1 mg kg^{-1}), Se delays senescence and promotes the growth of ageing seedlings in lettuce (*Lactuca sativa* L.), ryegrass (*Lolium perenne* L.) (Hartikainen and Xue 1999; Xue et al. 2001) and soybean (*Glycine max* L. Merr.) (Djanaguiraman et al. 2005). Furthermore, Pennanen et al. (2002) noted that Se increased starch granules in the chloroplasts of UV-(B)-stressed plants. Similar findings have been reported in Se-treated potato (*Solanum tuberosum* L.) by Turakainen et al. (2004). More recently, Malik et al. (2010) observed increased sucrose and starch accumulation in Se-treated mungbean (*Phaseolus aureus Roxb.*) due to enhanced activities of sucrose-synthesizing enzymes.

Studies on the effects of Se on plant-microbe interactions are very limited. In Se- hyperaccumulator *Brassica juncea* L., rhizosphere bacteria are found to enhance the accumulation and volatilization of Se (de Souza et al. 1999). However, to our knowledge, no study has been conducted on the effects of Se on *Rhizobiaceae* and other N_2-fixing bacteria. The main objective of this study was to investigate the effects of Se on carbohydrate metabolism, nodulation and growth in alfalfa. Our working hypothesis was that Se may enhance these processes through its positive effects on energy metabolism. The impact of Se on the activity of fructose 1,6-bisphosphatase (F1,6-BPase) and nitrogenase enzyme was investigated.

Materials and methods

Pot experiments

Pot experiments with alfalfa (*Medicago sativa* L. cv. Gareh-yondjeh, donated by the Agricultural Research Centre, Tabriz, Iran) were undertaken in the greenhouse

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[Department of Agricultural Sciences, University of Helsinki, Finland (EXP1) and the Department of Plant Sciences, University of Tabriz, Iran (EXP2)] to investigate the effects of Se on plant growth and nodule formation, as well as on N concentration and nitrogenase activity. Growing conditions in both facilities were maintained at 25/18 °C (day/night) temperature, relative humidity (RH) of 70–80 % and a photoperiod of 14/10 h (illumination/darkness). Natural light in the greenhouse was supplemented with 400 W high-pressure sodium lamps (Lucalox, LU 400/HO/T/40NG, Hungary) at a mean photosynthetic photon flux density (PPFD) of 350 μmol photons m^{-2} s^{-1}.

Alfalfa seeds were surface-sterilized with 5 % sodium hypochlorite and germinated (ten seeds per pot) in polyethene-lined 3 L pots containing 300 g of perlite (Norlite®, Nordisk Perlite ApS, Denmark). Pots and perlite were autoclaved before sowing. Experiments were carried out in a randomized split-plot design in four replications, with Se and N as the main treatments. On day 5 after germination, plants were thinned to six plants per pot and inoculated with a 1 mL suspension (10^8 CFU mL^{-1}) of *S. meliloti* strain (local strain from the Tabriz plain, Iran). The bacterial suspension was inoculated in yeast extract mannitol broth (YMB) and incubated on a rotary shaker (120 rpm) for 72 h. In EXP1, plants were weekly irrigated for 8 weeks with 150 mL of autoclaved half-strength (50 %) nutrient solutions (Hoagland and Arnon 1950) containing 0, 5 and 10 μmol L^{-1} Se was given as sodium selenate (Na_2SeO_4) with either 2 or 10 mmol L^{-1} N as $Ca(NO_3)_2$ and in EXP2 with nutrient solutions containing 0 or 1 μmol L^{-1} Se and 2 or 10 mmol L^{-1} N. The concentrations of the nutrients in the solution were later doubled (100 %) to promote vegetative growth. In addition, plants were watered weekly with 150 mL of deionised water (Millipore Milli Q/Q-POD ™, Ireland). Electrical conductivity (EC) of substrate measured during the experiment ranged from 2.6 to 5.8 dS m^{-1}.

Three harvesting schedules were implemented in EXP1: (a) first harvest at the early vegetative stage 1 week after Se application, (b) second harvest at the mid-vegetative stage 3 weeks after Se application, (c) third harvest at the late flower growth stage 8 weeks after Se application, hereafter referred to as H1, H2 and H3, respectively. Plants in EXP2 were harvested only once, 10 weeks after the Se application. Harvested plants were separated into leaves, stems and roots. The roots were carefully and thoroughly rinsed with deionised water and blotted dry with tissue paper. The number of active nodules (NN) and nodule fresh weights (NFW) were recorded in both experiments. The leaves, stems and roots were then dried at 70 °C for 72 h to a constant weight, and the dry weights (DW) were recorded. Samples were milled to fine particles in a miller with a 0.5-mm sieve (Retsch ZM 200, Germany) at 1,600 rpm. The total N and C contents of plants were determined by the Dumas combustion method using a Vario Max CN analyser (Elementar GmbH, Germany). Total C and N were expressed as percentage weight of sample.

Hydroponic experiments

Two separate hydroponic experiments (EXP 3 and 4) were carried out with non-inoculated seeds of alfalfa in a growth chamber at the Department of Agricultural Sciences, University of Helsinki, Finland. These experiments focused on the effects of Se on carbohydrate metabolism with an emphasis on F1,6-BPase activity. The experiments were conducted in a completely randomized factorial design with Se and N as the main treatments with four replicates. Experimental conditions in the growth chamber (Huurre Model M900 X 2000RT, Finland) were similar to the greenhouse conditions. Conditions were continuously monitored throughout the experiments. Surface-sterilized seeds were germinated in perlite moistened with $CaSO_4$ solution (0.05 mmol L^{-1}). Seven-day- old alfalfa seedlings were then pre-cultured for 2 weeks in 50 % aerated nutrient solution (Hoagland and Arnon 1950). Thereafter, plants were transferred into 1 L plastic pots covered with aluminium foil containing 100 % aerated nutrient solution of pH 5.8–6.0 with N as $Ca(NO_3)_2$ (2 and 10 mmol L^{-1}) and Se (0, 5, 10 and 15 μmol L^{-1} given as Na_2SeO_4). Nutrient solutions were changed every 72 h (EXP3). The plants used for the assay of F1,6-BPase activity were not supplemented with Se until harvest, as the sampling was done 0.5–6 h after Se application (EXP4).

Plants were sampled at the late-vegetative growth stage after a four-week Se supplementation with 100 % nutrient solution (EXP3). The fresh weights (FW) of the harvested plants were recorded, and subsamples were lyophilized at −70 °C (CHRiST TM GAMMA 1-16LSC/GAMMA 2-16LSC, Germany) for 72 h. Dry weights of the lyophilized subsamples were recorded and they were stored separately at

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−20 °C. For sugar, starch and Se analysis, they were milled into fine particles with an oscillating mill (MM 400 Retsch, Germany) at a frequency of 28/s and time of 4×30 s.

For the analysis of F1,6-BPase, two upper leaflets of alfalfa grown in 100 % nutrient solution for four weeks were sampled at five different time-points (0.5, 1, 2, 4 and 6 h) after the Se application (EXP4). The samples were immediately stored in liquid N_2 at −80 °C for the enzyme assay.

Analytical procedures

Carbohydrate analyses

Total soluble sugars (SS) were determined in two replicates per sample (n=4) using the anthrone method according to Sanchez et al. (1998), with some modifications. Soluble sugars were extracted from 20 mg of milled lyophilized plant samples in 2 mL Eppendorf tubes with 2 mL of 70 % ethanol. The extracts were centrifuged (Microcentrifuge 5417R, Eppendorf AG, Germany) at 10,600 g for 15 min, and the supernatants were collected into glass tubes (16×120 mm). Extraction with ethanol was repeated twice and followed by extraction with 0.5 mL of deionised water. The pooled supernatants were mixed with 3.5 mL of chloroform, vortexed and allowed to settle for 1 h. The aqueous phase of the mixture was stored at −20 °C until use. For analyses, 1.25 mL of anthrone reagent (2 g anthrone in 1 L of 72 % H_2SO_4) was mixed with a 0.25 mL aliquot of extracts as well as standard glucose solutions. The mixture was vortexed and placed in a boiling water bath for 15 min and cooled in a water bath at room temperature. Absorbance of cooled extracts was measured at a wavelength (λ) of 620 nm in a UV-Visible Spectrophotometer (PharmaSpec UV-1700, Shimadzu, Germany). The concentration of soluble sugars was calculated from a standard curve plotted with known concentrations of glucose.

Starch concentration of plant samples was determined using the Total Starch Assay Procedure (Amyloglucosidase/α-amylase) method (Megazyme International, Ireland) (AOAC Official Method 2002). Starch concentration in the plant extracts was determined spectrophotometrically and calculated as described in the protocol.

Stromal fructose 1,6-bisphosphatase (F1,6-BPase) analysis

Fructose 1,6-bisphosphatase activity was determined according to Holaday et al. (1992). In the F1,6-BPase measurement, 1.0 mL of the final assay mixture contained, 50 mmol Tris–HCl (pH 8.0), 10 mmol $MgCl_2$, 1 mmol EDTA, 10 mmol DTT, 4 mmol Fru 1,6-P_2, 0.5 mmol $NADP^+$, 4 U of phosphoglucose isomerase (PGI) and 2 U of glucose-6-phosphate dehydrogenase (G6PDH). The reaction was initiated by adding the enzyme extract (200 μL). The mixture was incubated for 30 min at 25 °C, and absorbance was measured using the UV Probe-kinetics program in the Spectrophotometer (PharmaSpec UV-1700, Shimadzu, Germany) at 340 nm for 3 min. Blanks were measured using 1.0 mL of reaction mixture without the enzyme extract. Enzyme activity was expressed as change of absorbance.

Nitrogenase activity

Nitrogenase activity was measured using acetylene reduction assay (ARA) (Hardy et al. 1973). The detached root system was put in a glass jar (300 mL), and 10 % of the gas inside was replaced by acetylene. After a 24-h incubation at 27 °C, 700 μL of gas inside the jar was extracted with a hypodermic syringe, and the concentration of ethylene was analysed by gas chromatography model GOW-MAC equipped with an FID detector. A 99.5 % standard ethylene was diluted 1000 times and 700 μL was injected into the gas chromatograph. After ARA measurement, the roots were dried and the dry weight of nodules weighed. ARA is expressed as pmol ethylene formed per min per g DW of nodules or roots.

Determination of chlorophyll, carotenoids and gas exchange parameters

The concentrations of carotenoids and chlorophyll (Chl) *a* and *b* were determined from three fully expanded leaves collected from four plants in each treatment. Pigments were extracted in cold acetone and measured after 24 h in the dark at 4 °C (Lichtenthaler and Wellburn 1985). Gas exchange parameters (CO_2 assimilation, transpiration and stomatal conductance) were measured with a portable gas exchange system (LCA-4, ADC Bioscientific Ltd., UK). To ensure a

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uniform recording across all samples, gas exchange measurements were taken at ambient CO_2 concentration and 300 μmol m^{-2} s^{-1} of PPFD at the leaf surface.

Selenium analysis

For determination of Se, the plant samples were dried overnight at 70 °C and 0.2–0.5 g of samples was weighed in two replicates (n=3–4) into wet digestion tubes and mixed with 10 mL of a concentrated acid mixture (HNO_3-$HClO_4$-H_2SO_4 in a ratio of 2.5:1.5:1). The extracts were subjected to an ammonium pyrrolidine dithiocarbamate-methyl isobutyl ketone (APDC-MIBK) extraction procedure described by Kumpulainen et al. (1983). The Se concentration of the final solution was determined using AAS (Model 5100 Zeeman spectrometer, Perkin Elmer, USA) connected to a graphite furnace (Model HGA 600 Perkin Elmer, USA) and equipped with a model AS-60 autosampler (Perkin Elmer, USA). Accuracy of the measurements was verified using three in-house reference samples for every batch of Se analysis. The method used to determine the Se concentration in plant samples is described in detail by Keskinen et al. (2010). The Se concentration was expressed in μg g^{-1} (DW).

Statistical analysis

Statistical analysis of data was performed using PASW analytical software (SPSS Inc., Chicago, IL, USA; Version 18). Data was subjected to a two-way ANOVA comparing means of Se and N treatments. Significant means were separated using the Tukey-HSD method at a 5 % level of significance.

Results

Selenium concentration

In pot experiment 1 (EXP1), in low N-plants, the Se uptake and accumulation in leaves, stems and roots generally increased in proportion to the Se application level as well as the frequency of applications (Table 1). The higher Se application (10 μmol L^{-1}) increased Se concentration in mature shoots and roots about 40- and 76-fold, respectively. In adequate-N plants, however, the Se concentration did not increase linearly in response to elevated Se level. In hydroponic plants (EXP3), Se concentrations in stems and roots tended to increase with increasing Se level (Table 2).

Nodule formation and development

In EXP1, the effect of Se on nodulation changed with growth of plants. At the earlier growth stage (H2), in low-N-plants, the lower Se application (5 μmol L^{-1}) increased nodule number (NN) by 62 % as compared with the control. At week 8 (H3), the Se-induced effect on NN was no longer observed (Table 3). At the adequate N level, no differences between the Se treatments were seen at different growth stages. As for nodule fresh weight (NFW), the only significant Se-induced effect was seen at the later growth stage (H3), where the lower Se addition reduced NFW in the plants cultivated at the adequate-N-level (Table 3).

In EXP2, the low Se addition (1 μmol L^{-1}) significantly increased NN (about 40 %) as well as NFW (59 %) at the lower N level, whereas no effect was found in adequate-N plants (Table 4). At the low-N level, Se slightly increased also the nitrogenase activity, whereas no activity was detected in plants cultivated at the adequate-N level.

Growth, chlorophyll contents and gas exchange parameters

Selenium application (1 μmol L^{-1}) increased the net CO_2 assimilation rate by 43 % in plants supplemented with low N relative to control plants in EXP2 (Table 5). However, Se did not affect the assimilation rate in adequate-N plants. Interestingly, in these plants, Se increased Chl *a* but had no effect on Chl *b* or carotenoid contents, transpiration and stomatal conductance. On the other hand, the effect of Se on plant growth was inconsistent. Although in EXP1 there were non-significant increases in FW of plants (data not shown), Se increased the shoot DW in some adequate-N-plants at the earlier growth stage (H1). The results indicate that Se exerted a slightly negative effect on biomass production in mature plants (Table 6). No Se-induced changes in root biomass production were detected. Similar observations were also made in EXP2 and in the hydroponic experiments (data not shown).

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Table 1 Selenium concentration (μg g^{-1} DW) in the shoot and root tissues of inoculated alfalfa cultivated at low N (2 mmol L^{-1}) and adequate N (10 mmol L^{-1}) and different Se (0, 5 and 10 μmol L^{-1}) concentrations and sampled at week 8 (H3) in EXP1

N level	Se addition	Shoots	Roots
2	0	0.03±0.0 ^{c}	0.05±0.0 ^{b}
	5	0.11±0.1 ^{b}	1.10±0.1 ^{b}
	10	1.21±0.1 ^{a}	3.83±0.6 ^{a}
10	0	0.07±0.0 ^{b}	0.35±0.1 ^{b}
	5	1.15±0.1 ^{a}	5.06±0.6 ^{a}
	10	1.04±0.2 ^{a}	3.80±0.4 ^{a}

Data are means ± SE; n=3–4. Values in a column within each N treatment with same letters are not significantly different at $p \leq 0.05$. Columns are tested separately

Nitrogen metabolism

In EXP1, Se exerted no effect on the C concentration of the shoots or roots of alfalfa (Table 7). Even though the higher Se level (10 μmol L^{-1}) significantly elevated the N concentration in shoots, this change was not reflected in the C/N ratio. Furthermore, in EXP2, Se had a slightly negative effect on free amino acids in mature plants (data not shown).

Carbohydrate concentration and F1,6-BPase activity

In the 4-week hydroponic experiment (EXP3), soluble sugars (SS) in leaves (p <0.001) and stems (p <0.01) increased in response to Se additions (Fig. 1). The only exceptions were the low-N plants, where Se had no effect at the lowest level, whereas at the highest level (15 μmol L^{-1}), it increased SS in the leaves by 46 %. Interestingly, in the leaves of adequate-N plants, the Se-induced increase was most significant (45 %) at the lowest Se level (5 μmol L^{-1}) compared with the control. As for roots, the impact of Se was in reverse relation to the N level. The higher Se levels (10 and 15 μmol L^{-1}) markedly elevated SS in the low-N plants, but drastically lowered these in the adequate-N plants.

The Se-induced changes in starch concentrations (Fig. 2) were less pronounced than those in SS. At the low-N level, Se applications of 10 and 15 μmol L^{-1} increased starch concentrations in leaves by 55–60 % compared with control plants. At the adequate-N level, only the highest Se application enhanced starch synthesis (about 34 %). In roots, Se exerted no effect on starch concentrations in adequate-N plants, while in the low-N plants, the highest Se level increased starch concentrations by (34 %) compared with control plants.

Furthermore, Se exerted a positive effect on stromal F1,6-BPase enzyme activity with time (EXP4, Fig. 3). The highest Se level (15 μmol L^{-1}) increased the enzyme activity about 50 % after 0.5 h compared with controls. The activity decreased slightly below the control value by 2 h, but increased to about 33 % of the control value by 6 h.

Table 2 Selenium concentration (μg g^{-1} DW) in the leaf, stem and root tissues in alfalfa supplied with different N (2 and 10 mmol L^{-1}) and Se (0, 5 10 and 15 μmol L^{-1}) concentrations for 4 weeks in hydroponics medium (EXP3)

N level	Se addition	Leaves	Stems	Roots
2	0	0.02±0.1 ^{b}	2.06±0.1 ^{d}	6.02±0.1 ^{c}
	5	3.48±0.4 ^{a}	18.42±0.1 ^{c}	90.89±2.9 ^{b}
	10	3.82±0.4 ^{a}	42.49±1.7 ^{b}	140.87±7.2 ^{a}
	15	4.40±0.2 ^{a}	55.93±6.6 ^{a}	156.66±21.5 ^{a}
10	0	0.09±0.1 ^{b}	1.70±0.1 ^{c}	5.01±0.1^{d}
	5	3.60±0.6 ^{a}	13.62±0.4 ^{b}	46.44±7.1 ^{c}
	10	3.70±0.4 ^{a}	33.88±2.5 ^{a}	148.56±0.5 ^{b}
	15	3.72±0.1 ^{a}	36.30±2.1 ^{a}	210.44±8.1 ^{a}

Data are means ± SE; n=3–4. Values in a column within each N treatment with same letters are not significantly different at $p \leq 0.05$. Columns are tested separately

Discussion

Selenium non-accumulators are generally considered sensitive to high Se concentrations (greater than 25 μg Se g^{-1} DM), whereas Se-accumulators can contain thousands of milligrams of Se kg^{-1} DM (Terry et al. 2000). As expected, the plants cultivated in perlite had far lower Se concentration (up to 1.21 μg g^{-1} DW in shoots) than those cultivated in hydroponics (up to 4.4 and 55.93 μg g^{-1} DW in leaves and stems, respectively). Although non-accumulators are sensitive to high Se concentration, they can tolerate and accumulate high concentrations without causing phytotoxicity in plants (Rani et al. 2005). Plants have different capacities to accumulate and partition Se. This depends on Se form and concentration, and the presence of competing ions (Zayed et al. 1998; Terry et al. 2000).

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Table 3 Nodule number (NN) and nodule fresh weight (NFW, mg) of inoculated alfalfa cultivated at low N (2 mmol L^{-1}) and adequate N (10 mmol L^{-1}) and different Se (0, 5 and 10 μmol L^{-1}) concentrations and sampled at week 3 (H2) and 8 (H3) in EXP1

N level	Se addition	NN		NFW	
		H2	H3	H2	H3
2	0	377.7±109.2 [b]	239.6±26.3 [a]	34.9±15.9 [a]	555.1±31.9 [a]
	5	984.5±356.7 [a]	282.1±20.2 [a]	61.8±23.8 [a]	634.8±19.3 [a]
	10	352.4±108.0 [b]	280.7±18.1 [a]	19.0±3.8 [a]	590.4±59.3 [a]
10	0	367.2±64.9 [a]	205.7±17.2 [a]	38.0±3.5 [a]	690.0±10.0 [a]
	5	305.3±132.2 [a]	220.0±35.1 [a]	28.8±19.6 [a]	577.9±35.2 [b]
	10	290.0±79.2 [a]	220.8±11.4 [a]	23.6±6.8 [a]	618.5±19.0 [ab]

Data are means ± SE; n=3–4. Values in a column within each N treatment with same letters are not significantly different at $p \leq 0.05$. Columns are tested separately

Several studies have shown that, selenate is efficiently translocated from roots to vegetative parts (de Souza et al. 1998; Pilon-Smits et al. 1998; Terry et al. 2000; Cartes et al. 2005; Sors et al. 2005). Wan et al. (1988) demonstrated that selenate was more efficiently translocated from the roots to shoots in alfalfa. However, in our study, Se was accumulated in the roots and the translocation to shoots seems restricted (Tables 1 and 2). This is in agreement with the findings of Williams and Mayland (1992), who showed that Se translocation from roots to vegetative parts, can be inefficient in non-accumulators. The low shoot: root Se concentration in our study, could not be explained by dry matter dilution, owing to decreases in the shoot biomass (Table 6). The reasons for the decreased translocation of selenate from roots to vegetative parts, thus, remain unclear. However, it is possible that, trace amounts of Se may have adhered to the root surface rather than being taken up, despite careful washing of the roots.

Table 4 Nodule fresh weights (NFW, mg), nodule number (NN) and nitrogenase activity (pmol/gNDW/min) in inoculated alfalfa cultivated at low N (2 mmol L^{-1}) and adequate N (10 mmol L^{-1}) and different Se (0, and 1 μmol L^{-1}) concentrations and sampled at week 10 in EXP2

N level	Se addition	NN	NFW	Nitrogenase activity
2	0	133±28 [b]	275±43 [b]	1340±234 [a]
	1	222±45 [a]	669±107 [a]	1422±341 [a]
10	0	83±5 [a]	52±8 [a]	0
	1	95±9 [a]	62±12 [a]	0

Data are means ± SE; n=3–4. Values in a column within each N treatment with same letters are not significantly different at $p \leq 0.05$. Columns are tested separately

Interestingly, it was also observed that Se accumulation was unexpectedly high in the roots than stems and leaves of control plants (Table 2). Although Se volatilization was not directly measured in our study, the high Se concentration in control plants may be attributable to contamination by volatile Se compounds. The rate of Se volatilization differs among plant species; reportedly being low in lettuce, intermediate in alfalfa and cucumber (*Cucumis sativus* L.) and high in broccoli (*Brassica oleracea* L.) (Terry et al. 1992; Zayed et al. 1998). Earlier studies on seasonal fluxes of Se in hyperaccumulators and non-accumulators indicate that these perennials can remobilize Se from vegetative and reproductive organs to the roots (Galeas et al. 2007; Pilon-Smits and Le Duc 2009). But Se uptake and partitioning in plants may also be influenced by plant species and developmental stage (Williams and Mayland 1992; Terry et al. 2000) and genetic traits (Banuelos et al. 2002). On the other hand, it may be necessary to separate the control plants from Se-treated plants in order to minimize possible Se contamination (Lyons et al. 2009).

In the present study, Se had beneficial effects on carbohydrate metabolism (SS and starch) in the shoots and roots of plants. This is consistent with previous findings in potato (Turakainen et al. 2004) and mungbean (Malik et al. 2010). Selenium additions (10 and 15 μmol L^{-1}) enhanced SS and starch accumulation in the shoots and roots of low-N-plants (Figs. 1 and 2). But the effect in adequate-N-plants was inconsistent. Interestingly, carbohydrates accumulation in the roots was in reverse relation to the N

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Table 5 Net assimilation rate (A, μmol m^{-2} s^{-1}), transpiration (E, mmol m^{-2} s^{-1}), stomatal conductance (g_s, mol m^{-2} s^{-1}), chlorophyll and carotenoids concentrations (mg g^{-1} FW), in inoculated alfalfa cultivated at low N (2 mmol L^{-1}) and adequate N (10 mmol L^{-1}) and different Se (0 and 1 μmol L^{-1}) concentrations and sampled at week 10 in EXP2

N level	Se addition	*A*	*E*	*gs*	Chl *a*	Chl *b*	Carotenoids
2	0	5.18±0.80 [b]	1.03±0.59 [a]	0.33±0.09 [a]	2.90±0.39 [a]	1.01±0.20 [a]	185±38 [a]
	1	9.12±0.03 [a]	1.56±0.38 [a]	0.37±0.08 [a]	3.54±0.35 [a]	1.31±0.24 [a]	232±42 [a]
10	0	5.63±0.24 [a]	1.26±0.28 [a]	0.33±0.03 [a]	2.03±0.37 [b]	1.12±0.21 [a]	169±16 [a]
	1	6.28±0.34 [a]	1.30±0.43 [a]	0.33±0.06 [a]	3.57±0.33 [a]	1.33±0.23 [a]	243±24 [a]

Data are means ± SE; n=3–4. Values in a column within each N treatment with same letters are not significantly different at $p \leq 0.05$. Columns are tested separately

level. The efficient accumulation of carbohydrates in Se-treated plants may be explained by the increased net CO_2 assimilation in low-N-plants and increased chlorophyll *a* content in adequate-N-plants (Table 5). Earlier findings show similar Se effect on chlorophyll *a* and carotenoids in blue-green algae (*Spirulina platensis* L.) (Chen et al. 2008) and in chlorophyll biosynthesis in mungbeans (Padmaja et al. 1990).

Selenium also altered F1,6-BPase activity in plants (Fig. 3). In order to assess the physiological effect of Se on the enzyme, we measured the kinetic parameters at different time points after Se application. Marques and Anderson (1985) reported that F1,6-BPase activity follow the Michaelis-Menten kinetics and therefore, the formation of the enzyme-substrate complex may be time-dependent. Although, the activity of F1,6-BPase measured fluctuated at various time-points, there was a transient increase in enzyme activity. This may be attributable to the Se-evoked modulation of the ferrodoxin/thioredoxin system resulting in enhanced ATP synthesis and fructose-6-phosphate formation for starch biosynthesis in chloroplasts (reviewed in Buchanan and Balmer 2005).

The efficient accumulation of carbohydrates in the shoots and roots of plants may have provided the energy substrates for initial nodulation in alfalfa (Tables 3 and 4; Figs. 1 and 2). This supports our hypothesis that, Se up-regulates carbohydrate metabolism with synergistic effects on nodulation. Our results indicate that, when added at low concentrations, both Se and N may exert some stimulatory effects on nodulation (EXP1 and EXP2). Although, low Se additions (1 μmol L^{-1} and 5 μmol L^{-1}) enhanced NN, high Se (10 μmol L^{-1}) had no significant effect on NN at all N-levels. Furthermore, NN decreased in matured plants in both experiments. This phenomenon can be explained by feedback regulation of nodulation by rhizobia in alfalfa (Caetano-Anolles and Bauer 1988) and the long-term effects of N (Carrol and Mathews 1990; Naisbitt and Sprent 1993).

However, Se effect on NFW was not well-defined. Even though Se enhanced NFW in low-N-plants, no

Table 6 Dry weights (DW) (g) of shoots and roots of inoculated alfalfa cultivated at low N (2 mmol L^{-1}) and adequate N (10 mmol L^{-1}) and different Se (0, 5 and 10 μmol L^{-1}) concentrations and sampled at week 1 (H1), 3 (H2) and 8 (H3) in EXP1

N	Se	H1		H2		H3	
Level	Addition	Shoot	Root	Shoot	Root	Shoot	Root
2	0	0.024±0.1[a]	0.008±0.1[a]	0.089±0.1[a]	0.068±0.1[a]	6.94±0.1[a]	2.79±0.5[a]
	5	0.023±0.1[a]	0.007±0.1[a]	0.068±0.1[a]	0.050±0.1[a]	6.71±0.1[a]	2.36±0.2[a]
	10	0.022±0.1[a]	0.007±0.1[a]	0.068 ±0.1[a]	0.039±0.1[a]	6.15±0.1[a]	3.11±0.2[a]
10	0	0.011±0.1[b]	0.004±0.1[a]	0.091±0.1[a]	0.089±0.1[a]	8.00±0.1[a]	4.57±0.1[a]
	5	0.026±0.2[a]	0.006±0.1[a]	0.086 ±0.1[a]	0.073±0.1[a]	7.06±0.5[a]	3.99±0.3[a]
	10	0.018±0.1[ab]	0.007±0.1[a]	0.075±0.1[a]	0.112±0.1[a]	6.76±0.3 [a]	4.44±0.7[a]

Data are means ± SE; n=3–4. Values in a column within each N treatment with same letters are not significantly different at $p \leq 0.05$. Columns are tested separately

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Table 7 Carbon (C % DW) and nitrogen (N % DW) concentrations and carbon:nitrogen (C/N) ratio of inoculated alfalfa cultivated at low N (2 mmol L^{-1}) and adequate N (10 mmol L^{-1}) and different Se (0, 5 and 10 μmol L^{-1}) concentrations and sampled at week 8 (H3) in EXP1

N	Se	C %		N %		C/N	
Level	Addition	Shoot	Root	Shoot	Root	Shoot	Root
2	0	42.0±0.1 ^{a}	40.8±1.2 ^{a}	1.9±0.1 ^{ab}	2.0±0.0 ^{a}	138.8±7.2 ^{a}	127.2±2.2 ^{a}
	5	41.9±0.3 ^{a}	41.0±0.3 ^{a}	1.6±0.0 ^{b}	1.8±0.2 ^{a}	130.2±5.6 ^{a}	128.6±9.5 ^{a}
	10	41.9±0.1^{a}	41.3±0.3 ^{a}	2.1±0.1 ^{a}	2.0±0.1 ^{a}	139.6±8.5 ^{a}	134.0±3.5 ^{a}
10	0	41.8±0.1 ^{a}	41.0±0.5 ^{a}	1.7±0.1 ^{b}	2.0±0.1 ^{ab}	162.9±5.3 ^{a}	135.1±3.8 ^{a}
	5	42.0±0.1 ^{a}	41.8±0.1 ^{a}	2.1±0.1 ^{a}	1.9±0.1 ^{b}	154.8±7.6 ^{a}	126.4±6.6 ^{a}
	10	41.7±0.1 ^{a}	41.4±0.4 ^{a}	2.0±0.1 ^{a}	2.2±0.1 ^{a}	140.2±8.2 ^{a}	115.4±3.0 ^{a}

Data are means ± SE; n=3–4. Values in a column within each N treatment with same letters are not significantly different at $p \leq 0.05$. Columns are tested separately

such effect was seen in adequate-N-plants (EXP2; Table 4), and also at all N-levels in EXP1 (Table 3). Nitrogenase activity (Table 4), proteins and amino acids (data not shown) and N concentrations in plants (Table 7) were not significantly affected by Se. Although previous studies have emphasized the important contributions of nitrogenase and proteins/amino acids to nodulation and N_2 fixation (Dénarié et al. 1993; Cooper 2007), to our knowledge, no studies exist on the effect of Se on N_2 fixation. More detailed studies should be conducted to fully understand the effect of Se on the biochemical and physiological processes involved in N_2 fixation.

In contrast to higher accumulation of carbohydrates in the shoots and roots, growth (DM accumulation) was not affected by Se (Tables 6 and 7; Figs. 1 and 2). This suggests that photoassimilates were not efficiently translocated and utilized in biomass production. This is inconsistent with previous findings by Turakainen et al. (2004) in potato and Malik et al. (2010) in mungbean where increases in carbohydrates resulted in higher above-ground biomass. Nonetheless, our results agree with previous findings in alfalfa (Broyer et al. 1966), ryegrass (Hartikainen et al. 1997) and sorghum (Djanaguiraman et al. 2010) where low Se applications had no significant effects on growth. The translocation of photoassimilates

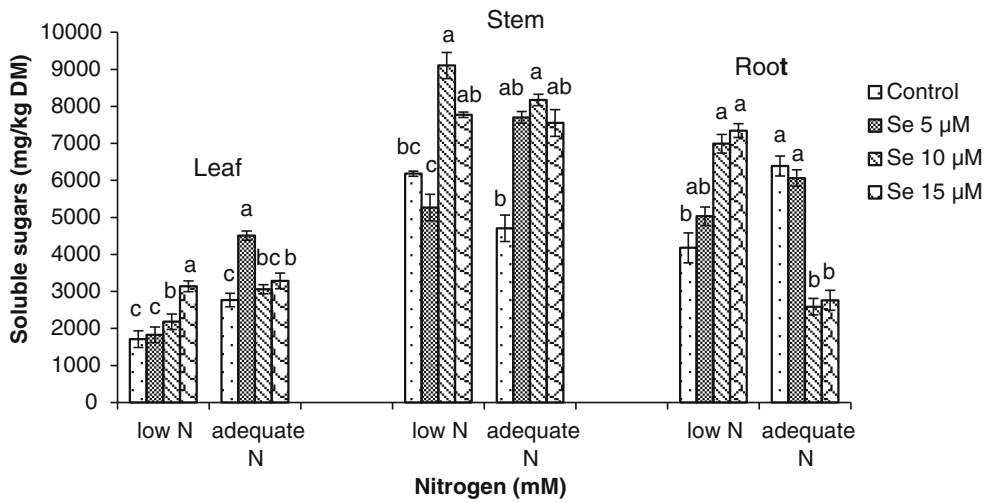

Fig. 1 Soluble sugars concentration (mg/kg DM) of alfalfa supplemented with different N (low N – 2 mM; adequate N – 10 mM) and Se (0, 5, 10 and 15 μmol L^{-1}) for 4 weeks in hydroponics (EXP3). Data are means ± SE; n=4. Means with same letters within each N treatment are not significantly different at $p \leq 0.05$

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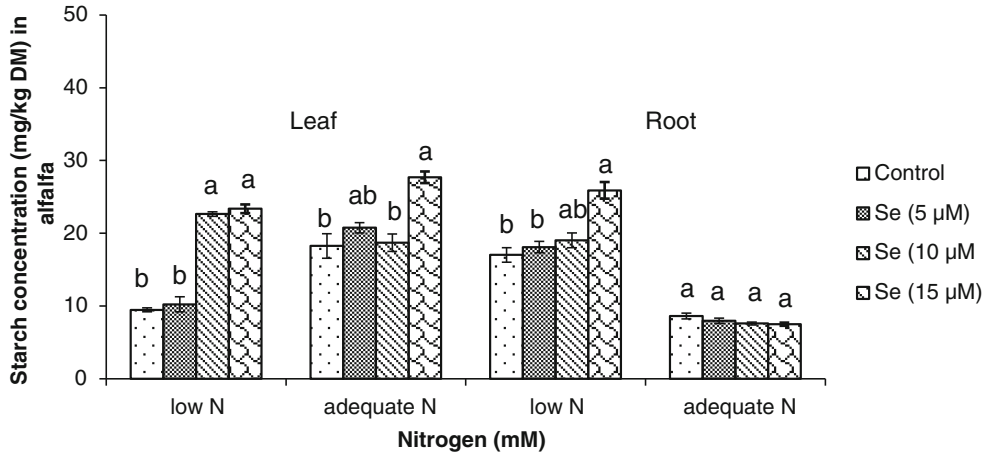

Fig. 2 Starch content (mg/kg DM) in leaves and roots of alfalfa supplemented with different N (low N – 2 mM; adequate N – 10 mM) and Se (0, 5, 10 and 15 μmol L^{-1}) for 4 weeks in hydroponics (EXP3). Data are means ± SE; n=4. Means with same letters within each N treatment are not significantly different at $p \leq 0.05$

from source organs to sink organs is complex and may also be influenced by net CO_2 assimilation and the sink-strength (reviewed in Gifford and Evans 1981; Long and Drake 1992), xylem/phloem transporters and feedback control of photosynthesis (reviewed in Geiger and Servaites 1994; Paul and Foyer 2001).

However, growth-promoting Se effects have been reported in lettuce (Xue et al. 2001), ryegrass (Hartikainen et al. 1997; Hartikainen and Xue 1999) and soybean (Djanaguiraman et al. 2005). These effects have been attributed to the antioxidation function of Se (Hartikainen et al. 2000; Djanaguiraman et al. 2005). Overall, these studies reveal the doubled-edged nature of this trace element.

Conclusion

Our study provides indication on the positive effects of Se on carbohydrates in alfalfa. Selenium enhanced

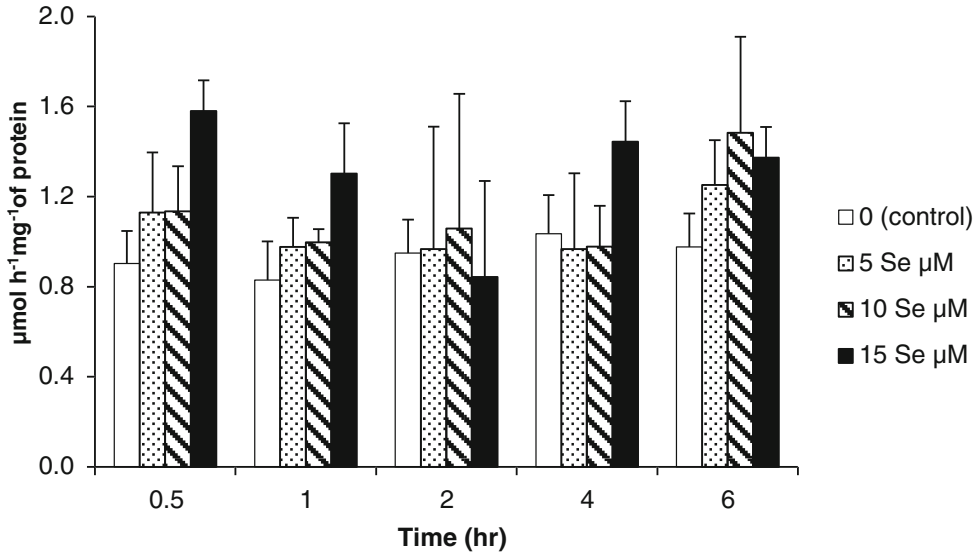

Fig. 3 Fructose 1,6-bisphosphatase (F1,6-BPase) activity (μmol h^{-1} mg^{-1} of protein) in low N (2 mmol L^{-1}) alfalfa grown hydroponically (EXP4). The activity changes were monitored 0, 0.5, 1, 2, 4 and 6 h after transfer to nutrient solution supplemented with 0, 5, 10 or 15 μmol L^{-1} Se. Each *bar* is the mean of four replicates ± standard error

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soluble sugars and starch accumulation in the shoots and roots of young plants. This was supported by the stimulatory Se effects on photosynthesis and F1,6-BPase activity. In addition, the efficient accumulation of carbohydrates may also have provided the energy substrates for initial nodulation in alfalfa. Although, Se effect on nodule fresh weight (NFW), nitrogenase activity and N concentration in plants was not significant, our results suggest that, Se via altered redox potential or antioxidation function may have some stimulatory effects on nodulation. Little is known about the role of Se in N_2-fixation, hence the need for more detailed studies to fully understand the effects of Se on rhizobia and nitrogenase in N_2-fixation. In our study, Se had no significant effect on growth. In matured plants, it had a slightly negative effect on fresh weights and biomass accumulation. This may be attributable to reduced translocation and utilization of photoassimilates in the matured plants. Overall, the data clearly suggest that the effects of Se are dependent on the Se concentration and developmental stage of the plant.

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References

AOAC (2002) Official Methods of Analysis of AOAC International. Volume I & II. 17th edition. AOAC International. 1st revision. Gaithersburg, MD, USA, Association of Analytical Communities

Banuelos GS, Vickerman DB, Trumble JT, Sharmon MC, Davis CD, Finley JW, Maylands HF (2002) Biotransfer possibilities of selenium from plants used in phytoremediation. Int J Phytorem 4:315–329

Broyer TC, Lee DC, Asher CJ (1966) Selenium Nutrition of Green Plants. Effect of selenite supply on growth and selenium content of alfalfa and subterranean clover. Plant Physiol 41:1425–1428

Buchanan BB, Balmer Y (2005) Redox regulation: a broadening horizon. Annu Rev Plant Biol 56:187–220

Caetano-Anolles G, Bauer WD (1988) Feedback regulation of nodule formation in alfalfa. Planta 175:546–557

Carrol BJ, Mathews A (1990) Nitrate inhibition of nodulation in legumes. In: Gresshoff PM (ed) Molecular Biology of Symbiotic Nitrogen Fixation. CRC Press Inc, pp 159–180

Cartes P, Gianfera L, Mora ML (2005) Uptake of selenium and its antioxidant activity in ryegrass when applied as selenate and selenite forms. Plant Soil 276:359–367

Chen TF, Zheng WJ, Wong YS, Yang F (2008) Selenium-induced changes in activities of antioxidant enzymes and content of photosynthetic pigments in *Spirulina platensis*. J Integr Plant Biol 50(1):40–48

Cooper JE (2007) Early interactions between legumes and rhizobia: disclosing complexity in a molecular dialogue. J Appl Microbiol 103:1355–1365

Coppinger RJ, Diamond AM (2001) Selenium deficiency and human disease. In Hatfield DL (ed) Selenium: its molecular biology and role in human health. Kluwer Academic Publisher, pp 219–331

de Souza MP, Pilon-Smits EAH, Lytle CM, Hwang S, Tai J, Honma TSU, Yeh L, Terry N (1998) Rate-limiting steps in selenium assimilation and volatilization by Indian mustard. Plant Physiol 117:1487–1494

de Souza MP, Chu D, Zhao M, Zayed AM, Ruzin SE, Schichnes D, Terry N (1999) Rhizosphere bacteria enhance selenium accumulation and volatilization by Indian mustard. Plant Physiol 119:565–573

Dénarié J, Debellé F, Truchet G, Promé JC (1993) Rhizobium and legume nodulation: A molecular dialogue. In: Palacios R, Mora J, Newton WE (eds) New horizons in nitrogen fixation. Kluwer, Dordrecht, pp 19–30

Djanaguiraman M, Durga Devi D, Shanker AK, Sheeba JA, Bangarusamy U (2005) Selenium—an antioxidative protectant in soybean during senescence. Plant 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 Physiol Biochem 48:999–1007

Fisher RF, Long SR (1992) *Rhizobium*-plant signal exchange. Nature 357:655–660

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 Phytol 173:517–525

Geiger DR, Servaites JC (1994) Diurnal regulation of photosynthetic carbon metabolism in C3 plants. Annu Rev Plant Physiol Plant Mol Biol 45:235–256

Gifford RM, Evans LT (1981) Photosynthesis, carbon partitioning, and yield. Annu Rev Plant Physiol 32:485–509

Hardy RWF, Burns RC, Holsten RD (1973) Applications of the acetylene-ethylene assay for measurements of nitrogen fixation. Soil Biol Biochem 5:47–81

Hartikainen H (2005) Biogeochemistry of selenium and its impact on food chain quality and human health. J Trace Elem Med Biol 18:309–318

Hartikainen H, Xue T (1999) The promotive effect of selenium on plant growth as trigged by ultraviolet irradiation. J Environ Qual 28:1272–1275

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. Agr Food Sci Finl 6:381–387

Hartikainen H, Xue T, Piironen V (2000) Selenium as an antioxidant and prooxidant in ryegrass. Plant Soil 225:193–200

Hawker S (1985) Sucrose. In: Dey PM, Dixon RA (eds) Biochemistry of storage carbohydrates in green plants. Academic Press, London, pp 1–51

Author's personal copy

Hawrylak-Nowak B (2009) Beneficial effects of exogenous selenium in cucumber seedlings subjected to salt stress. Biol Trace Elem Res 132:259–269

Hoagland DR, Arnon DI (1950) The water culture method for growing plants without soil. California Agricultural Experiment Station Circular 347. Berkely, CA, pp 1–39

Holaday A, Martindale W, Alred R, Brooks A, Leegood R (1992) Changes in activities of enzymes of carbon metabolism in leaves during exposure of plants to low temperature. Plant Physiol 98:1105–1114

Joshi PA, Caetano-Anolles G, Graham ET, Gresslsoff PM (1993) Ultrastructure of transfer cells in spontaneous nodules of alfalfa (*Medicago sativa*). Protoplasma 172:64–76

Keskinen R, Turakainen M, Hartikainen H (2010) Plant availability of soil selenate additions and selenium distribution within wheat and ryegrass. Plant Soil 333:301–313

Kumpulainen J, Raittila AM, Lehto J, Koivistoinen P (1983) Electrothermal atomic absorption spectrometric determination of selenium in foods and diets. J Assoc Off Anal Chem 66:1129–1135

Lichtenthaler HK, Wellburn AR (1985) Determination of total carotenoids and chlorophylls *a* and *b* of leaf in different solvents. Biochem Soc Trans 11:591–592

Long SP, Drake BG (1992) Photosynthetic CO_2 assimilation and rising atmospheric CO_2 concentration. In: Baker NR, Thomas H (eds) Topics in Photosynthesis Research. Elsevier, pp 69–104

Lyons GH, Genc Y, Soole K, Stangoulis JCR, Liu F, Graham RD (2009) Selenium increases seed production in Brassica. Plant Soil 318:73–80

Malik JA, Kumar S, Thakur P, Sharma S, Kaur N, Kaur R, Pathania D, Bhandhari K, Kaushal N, Singh K, Srivastava A, Nayyar H (2010) Promotion of Growth in Mungbean (Phaseolus aureus Roxb.) by Selenium is Associated with Stimulation of Carbohydrate Metabolism. Biol Trace Elem Res 143:530–539

Marques IA, Anderson LE (1985) Changing Kinetic Properties of Fructose-1,6-bisphosphatase from Pea Chloroplasts during Photosynthetic Induction. Plant Physiol 77:807–810

Michaud R, Lehman WF, Rumbaugh MD (1988) World distribution and historical development. In Hanson AA, Barnes DK, Hill Jr. RR (eds) Alfalfa and Alfalfa Improvement. Academic Press, pp 25–91

Naisbitt T, Sprent JI (1993) The long-term effects of nitrate on the growth and nodule structure of the caesalpinioid herbaceous legume Chamaecrista fasciculata Michaux. J Exp Bot 44:829–836

Nap J-P, Bisseling T (1990) Developmental biology of a plant prokaryote symbiosis: the legume root nodule. Science 250:948–954

Padmaja K, Prasad DD, Pradad AR (1990) Selenium as a novel regulator of porphyrin biosynthesis in germinating seedlings of mung bean (*Phaseolus vulgaris* L.). Biochem Int 22:441–446

Paul MJ, Foyer CH (2001) Sink regulation of photosynthesis. J Exp Bot 52(360):1383–1400

Pennanen A, Xue T, Hartikainen H (2002) Protective role of selenium in plant subjected to severe UV irradiation stress. J Appl Bot 76:66–76

Pilon-Smits EAH, Le Duc DL (2009) Phytoremediation of selenium using transgenic plants. Curr Opin Biotechnol 20:207–212

Pilon-Smits EAH, de Souza MP, Lytle CM, Shang C, Lugo T, Terry N (1998) Short communications. Selenium volatilization and assimilation by hybrid poplar (*Populus tremula x alba*). J Exp Bot 49:1889–1892

Pilon-Smits EAH, Quinn CF, Tapken W, Malagoli M, Schiavon M (2009) Physiological functions of beneficial elements. Curr Opin Plant Biol 12:267–274

Rani N, Dhillon KS, Dhillon SK (2005) Critical levels of selenium in different crops grown in an alkaline silty loam soil treated with selenite-Se. Plant Soil 277:367–374

Sánchez FJ, Manzanares M, de Andres EF, Tenorio JL, Ayerbe L (1998) Turgor maintenance, osmotic adjustment and soluble sugar and proline accumulation in 49 pea cultivars in response to water stress. Field Crops Res 59:225–235

Sors TG, Ellis DR, Salt DE (2005) Selenium uptake, translocation, assimilation and metabolic fate in plants. Photosynth Res 86:373–389

Stinner BR, Blair JM (1990) Ecological and agronomic characteristics of innovative cropping systems. In: Edwards CA et al (eds) Sustainable agricultural systems. St. Lucie Press, Florida, pp 123–140

Terry N, Carlson C, Raab TK, Zayed AM (1992) Rates of selenium volatilization among crop species. J Environ Qual 21:341–344

Terry N, Zayed A, De Souza MP, Tarun AS (2000) Selenium in higher plants. Annu Rev Plant Physiol Plant Mol Biol 51:401–432

Turakainen M, Hartikainen H, Seppänen MM (2004) Effects of selenium treatments on potato (*Solanum tuberosum* L.) growth and concentrations of soluble sugars and starch. J Agr Food Chem 52:5378–5382

United States Department of Agriculture Research Service (USDA 2007) National Nutrient Database Reference Release 21, Nutrient Data Laboratory Home Page http://www.ars.usda.gov.nutrient.da

Wan HF, Mikkelsen RL, Page AL (1988) Selenium uptake by some agricultural crops from Central California soils. J Environ Qual 17(2):269–272

White PJ, Broadley MR (2009) Biofortification of crops with seven mineral elements often lacking in human diets: iron, zinc, copper, calcium, magnesium, selenium and iodine. New Phytol 182:49–84

Williams MC, Mayland HF (1992) Selenium absorption by two-grooved milkvetch and western wheatgrass from selenomethionine, selenocystine, and selenite. J Range Manage 45:374–378

Xue T, Hartikainen H, Piironen V (2001) Antioxidative and growth-promoting effect of selenium in senescing lettuce. Plant Soil 237:55–61

Yao X, Chu J, Wang G (2009) Effects of selenium on wheat seedlings under drought stress. Biol Trace Elem Res 130:283–290

Zayed AM, Lytle CM, Terry N (1998) Accumulation and volatilization of different chemical species of selenium by plants. Planta 206:284–292