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ORIGINAL PAPER

ACCUMULATION OF SELECTED MACRONUTRIENTS AND TOLERANCE TOWARDS SELENIUM OF GARDEN PEA TREATED WITH SELENITE AND SELENATE*

Sabina Łukaszewicz[1], Barbara Politycka[1], Sylwester Smoleń[2]

[1] Department of Plant Physiology
Poznan University of Life Sciences, Poland
[2] Unit of Plant Nutrition
University of Agriculture in Cracow, Poland

ABSTRACT

Selenium (Se) applied at high concentrations can increase or decrease the concentrations of macronutrients in plants. The current knowledge of the interactions between Se and essential nutrients (except S) is insufficient and this problem should be investigated further. A laboratory study was conducted to evaluate the effects of Se on concentrations of selected macronutrients in garden pea (*Pisum sativum* L.). Pea seedlings were grown under growth chamber conditions in Hoagland's nutrient solution No 1. Se was supplied to the nutrient solution in the form of selenate or selenite at a concentration of 10, 20, 50 and 100 μmol dm^{-3} when pea seedlings were in the stage of the first pair of leaves. The length of roots as well the concentrations of Se and selected macronutrients (K, P, Ca, S and Mg) in roots and shoots were determined when the seedlings were in the stage of the third pair of leaves. Concentrations of Se and macronutrients were analysed on an inductively coupled plasma optical emission spectrometer (ICP-OES). Pea seedlings absorbed Se from the nutrient solution and accumulated it mainly in roots. More Se was accumulated when it was applied in the form of selenate than selenite. Under the influence of Se, a decrease in the accumulation of K, P, Ca, S and Mg in pea seedlings was found, with the exception of shoots treated with selenate, in which an increase in the concentrations of K, Ca and S was observed. Pea seedlings showed greater tolerance to selenate than selenite. Index of tolerance was calculated based on the length of roots. The novelty of the research lies in demonstrating the correlation between K and Ca concentrations and the tolerance of pea seedlings to Se.

Keywords: selenate, selenite, potassium, phosphorus, calcium, sulphur, magnesium, *Pisum sativum*, tolerance.

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prof. Barbara Politycka, Department of Plant Physiology, Poznań University of Life Sciences, Wołyńska 35, 60-637 Poznań, Poland, e-mail: barpolit@yahoo.pl

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INTRODUCTION

Selenium (Se) is an essential element for animals and humans but is not considered to be an essential nutrient for plants. This metalloid exerts dual effects on living organisms, namely it is beneficial at low concentrations, but toxic at high concentrations (Hartikainen et al. 2000, Combs 2001). Toxic levels of Se for plants vary from species to species (Kaur et al. 2014). Soils in some areas of North America, New Zealand, Australia, Asia, Europe and Africa are Se-deficient. It is reported that 15% of the global human population suffer from Se deficiency (Combs 2001, White, Broadley 2009). Since much of Se in human diet is derived directly or indirectly from plant food, tnumerous studies have focused on the biofortification of edible crops with this element. Inorganic fertilization is the most common practice to enhance the Se level in crop plants (Ros et al. 2016). Pea belongs to the group of crop plants with great potential for use in Se biofortification programs (Poblaciones et al. 2013), but the scientific basis of this solution is not well documented in the literature. Although Se biofortification has been introduced into agricultural practice, it should be used with great caution, since in contrast to most trace elements, the safety limit between deficiency and a toxic dose of Se is very small (Hartikainen et al. 2000). Moreover, it is difficult to predict the effects of selenium fertilization on the mineral status of the plant. Admittedly, Se fertilization increases the concentration of this trace element in plants, although biofortification should only be used if it has no negative effects on the uptake of essential nutrients. It is known that Se at higher doses can decrease or increase of their concentrations or have no effect. Nonetheless, the current knowledge of the interactions between Se and essential nutrients (except S) is insufficient and this problem should be further investigated (Longchamp et al. 2016, Domokos-Szabolcsy et al. 2017). Therefore, the main aim of the present study was to examine the effect of selenate and selenite application on macronutrient concentrations in garden pea (*Pisum sativum* L.) seedlings and the tolerance of this species towards Se. The research hypothesis assumes that Se affects the uptake of macronutrients and that there is Se tolerance in pea seedlings at a lower concentration of this element.

MATERIAL AND METHODS

Seeds of garden pea (*Pisum sativum* L.) *cv.* Akord were germinated for 4 days and then uniformly germinated seeds were transferred to containers with 4 dm^3 of aerated, modified full-strength Hoagland's nutrient solution No. 1 and grown for the next 4 days. The final concentrations of compounds in the nutrient solution were as follows: KH_2PO_4 – 1, KNO_3 – 5,

$Ca(NO_3)_2 \cdot 4H_2O$ – 5, $MgSO_4 \cdot 7H_2O$ – 2, $ZnSO_4 \cdot 7H_2O$ – 0.08, $MnSO_4 \cdot 5H_2O$ – 0.08, $CuSO_4 \cdot 5H_2O$ – 0.03, $(NH_4)_2Mo_7O_{24} \cdot 4H_2O$ – 0.02, H_3BO_3 – 0.05, NaFeEDTA – 0.07 (mmol dm^{-3}). Se was added to the nutrient solution as sodium selenite (Na_2SeO_3 $5H_2O$) or sodium selenate (Na_2SeO_4) at the concentrations of 10, 20, 50 and 100 μmol dm^{-3} when pea seedlings were in the stage of the first pair of leaves. Control seedlings were grown in nutrient solution without Se. The experiment was carried out in a controlled environment of a growth chamber with the following conditions: luminescent light with photon flux density of 135 μmol m^{-2} · s^{-1} (Philips lamps), a 14/10 day/night photoperiod, at 27/23°C day/night temperatures and relative humidity of about 60%. The experiment was repeated 3 times in 3 replications, each replication involving 1 container with 35 pea seedlings. Seedlings were grown in Hoagland's solution No. 1 with selenium for 7 days and when the plants were in the stage of the third pair of leaves, samples were collected.

The length of roots was measured as a parameter for the calculation of index of tolerance (TI) towards Se (Hawrylak-Nowak 2008). Results are the means of 300 seedlings. TI was calculated according to the equation:

$$TI = \frac{\text{the root length under Se treatment}}{\text{the root length in the control}} \cdot 100\%.$$

Air-dried 0.5 g samples of pea shoots and roots were digested at 200°C (15 min of warming plus 20 min of maintaining the set temperature) in 10 cm^3 of 65% super pure HNO_3 using a microwave system CEM MARS-5 Xpress. Samples were then transferred to the final volume of 25 cm^3 using double-distilled water. The analyses of Se, K, P, Ca, S and Mg concentrations in pea samples were conducted using an inductively coupled plasma optical emission Prodigy spectrometer (ICP-OES Teledyne Leeman Labs USA).

The results were subjected to statistical analysis using two-way analyses of variance and the significance of differences between means was estimated by the Duncan's test at the level of statistical significance at $\alpha \leq 0.05$. Moreover, regression analyses between K and Ca concentrations and the index of tolerance were conducted.

RESULTS AND DISCUSSION

Se concentration in roots (Figure 1*a*) and shoots (Figure 1*b*) of pea seedlings significantly increased with the increasing levels of applied Se. In seedlings treated with selenate, the Se concentration was higher than in seedlings treated with selenite. This disproportion grew as the Se concentration in the nutrient solution was higher. On average, a 10-fold difference in the total Se concentration was observed between selenate and selenite treatments. Our results resemble some previous studies which showed, for different plant

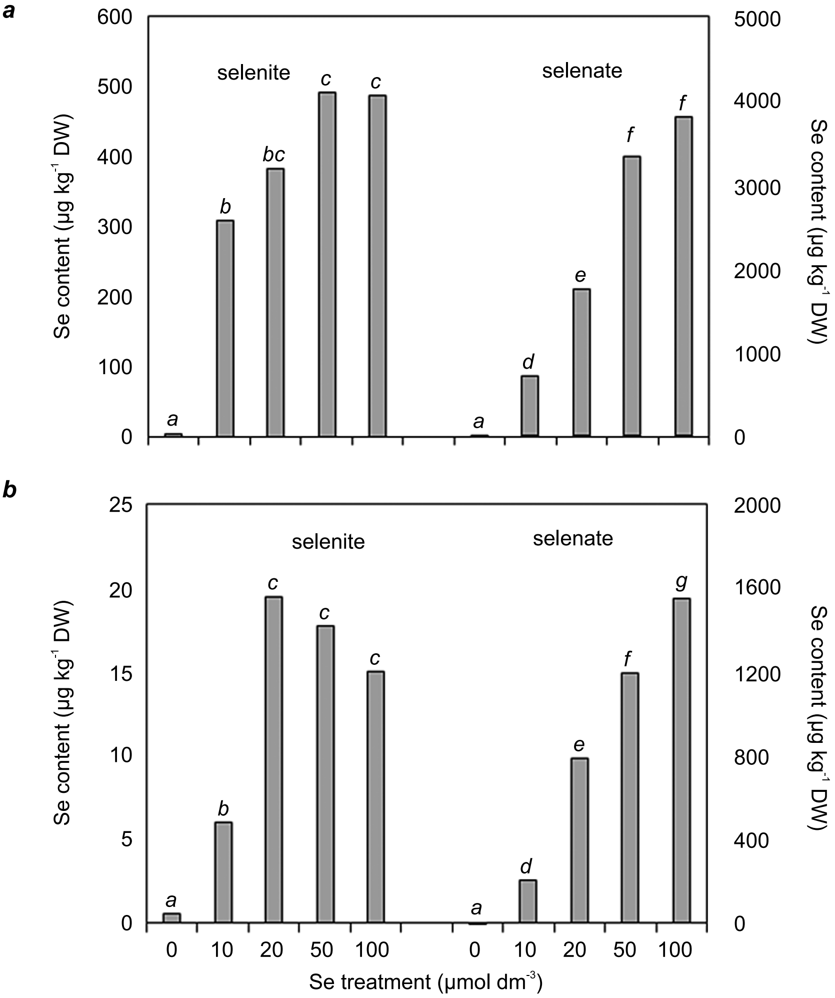

Fig. 1. Selenium concentration in roots (*a*) and shoots (*b*) of pea supplied with selenium as selenite and selenate. Bars marked with the same letters do not differ significantly at $\alpha < 0.05$.

species, that a higher concentration of Se was observed in plants treated with selenate than selenite (Cartes et al. 2005, de Souza et al. 2013, Garousi et al. 2016, Molnárová, Fargašová 2016). Regardless of the Se form, more Se accumulated in the roots than in the shoots of pea seedlings. The roots accumulated on average 96.62% of Se when supplied selenite and 78.14% when selenate had been added to the nutrient solution. Such high accumulation of Se in the roots of seedlings treated with selenate is contradictory to the reports of many authors, who found a much higher Se concentration in shoots in plants treated with selenate than selenite (de Souza et al. 2000, Hartikainen et al. 2000, Ramos et al. 2010). It is believed that selenate is taken up by roots actively through sulphate transporters and then it is quickly transported in the xylem to shoots. In contrast, selenite uptake occurs passively and only some is taken up actively by phosphate transporters. For this reason, less selenite is translocated into the xylem and most of it remains in roots, where it is readily converted into organic forms (DE SOUZA 2000, TERRY et al. 2000, LI et al. 2008, KOSTOPOULOU et al. 2010). However, results of numerous reports suggest that the distribution of Se applied as selenite or selenate between shoots and roots is dependent on plant species (ARVY 1993, HAWRYLAK-NOWAK 2013, HAWRYLAK-NOWAK et al. 2015, GAROUSI et al. 2016).

In our previous study conducted in the same experimental design, we found that Se application at a concentration of only 20 μmol-dm^{-3} caused a significant reduction in the dry weight of shoots (ŁUKASZEWICZ et al. 2018). In the current report, the length of roots in pea seedlings under an Se treatment is shown in Figure 2. Compared to the control, a stronger inhibitory effect on root elongation was exerted by selenite than selenate. In selenite-treated roots, significant growth inhibition was observed at the concentration of only 20 μmol Se dm^{-3}, and the effect was stronger with the increasing level of selenite in nutrient solution. The effect of selenate was different. Stimulation of root growth at the Se concentration of 20 μmol dm^{-3} was observed and a significant reduction of root length occurred only in seedlings treated with 100 μmol dm^{-3} selenate. It is well documented that the Se effect on plant growth is dependent on the Se chemical form, its concentration and sensitivity of plant species (MOLNÁROVÁ, FARGAŠOVÁ 2009, FILEK et al. 2010, MROCZEK-ZDYRSKA, WÓJCIK 2012, HAWRYLAK-NOWAK 2013, CHEN et al. 2014, HAWRYLAK-NOWAK et al. 2015, MOLNÁROVÁ, FARGAŠOVÁ 2016). The calculated index of tolerance clearly showed that pea seedlings have greater tolerance towards selenate than selenite (Figure 3). This is difficult to explain considering such high Se accumulation in the roots treated with selenate (Figure 1*a*). The phytotoxicity of Se is attributed to its pro-oxidative activity as well as to

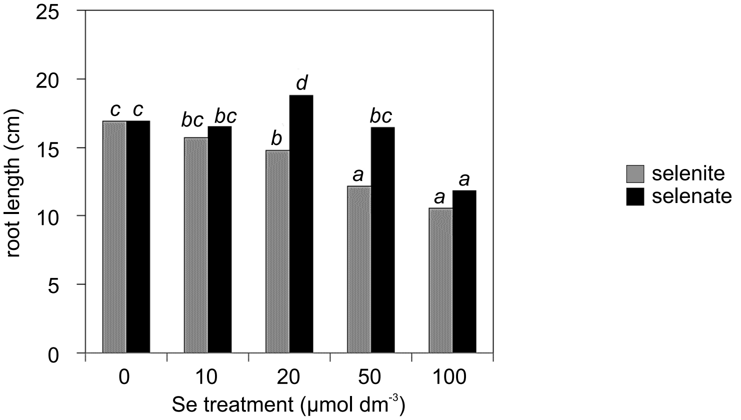

Fig. 2. Root length of pea seedlings supplied with selenium as selenite and selenate. Bars marked with the same letters do not differ significantly at $\alpha < 0.05$

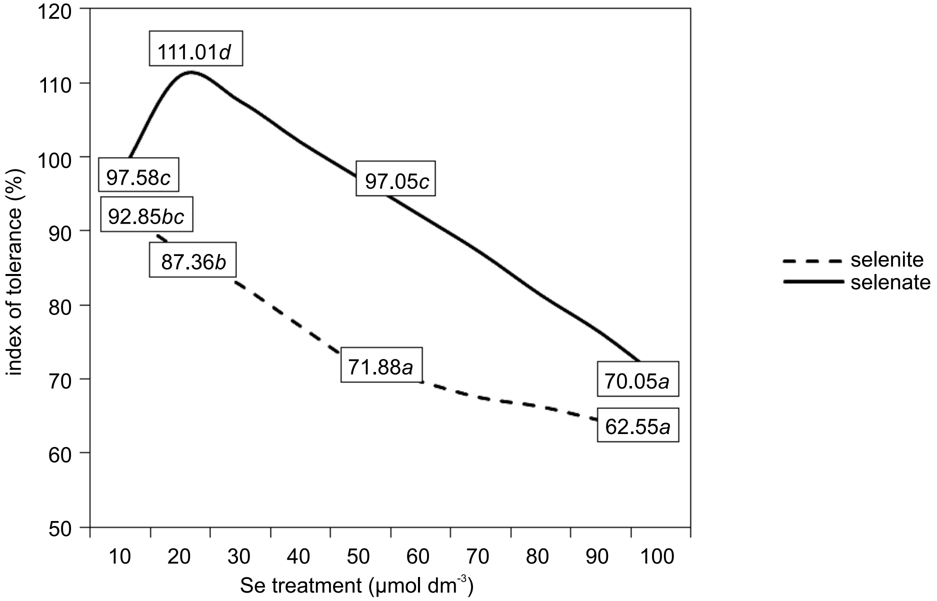

Fig. 3. Index of tolerance of pea supplied with selenium as selenite and selenate. Results marked with the same letters do not differ significantly at $\alpha < 0.05$

its incorporation into cysteine and methionine, and the non-specific selenoproteins formation (Terry 2000, White 2016, Kolbert et al. 2016). Selenite is rapidly converted into organic Se species and stored mainly in roots with limited translocation to shoots (Zayed et al. 1998, Li et al. 2008). Although it has not been proven for roots, it is likely that selenate was partly stored in vacuoles (Terry 2000, White 2016). Less tolerance of pea seedlings to selenite could have been caused by the more rapid conversion of selenite than selenate into selenoamino acids (Zayed et al. 1998), which may be then incorporated into plant proteins and cause toxicity to the plant. Great disproportion between Se accumulation (Figure 1) and tolerance (Figure 3) in pea seedlings treated with Se in the two forms can also be explained by an updated concept of the background mechanisms of Se toxicity presented by Kolbert et al. (2016), who suggested the inclusion of hormonal disturbances and generation of reactive nitrogen species. Lehotai et al. (2016) have discovered the generation of reactive oxygen species as well as reactive nitrogen species in pea seedlings treated with selenite. Kolbert et al. (2016) also postulate to include into the mechanism of Se toxicity the effect exerted by this element on the mineral nutrient status of plants.

Analysis of macronutrients showed that Se affected their concentrations in pea seedlings (Tables 1 and 2). Concentrations of K, Ca and Mg decreased compared to the control under the influence of the increasing Se concentration in roots of seedlings treated with Se in both forms and in shoots treated with selenite. A significant decrease was observed in all cases above the

Table 1

Concentrations of macronutrients in the roots of pea seedlings supplied with selenium as selenite and selenate

Treatment	K	P	Ca	S	Mg
	(mg g^{-1} DW)				
Control	54.10c*	4.261*ab*	5.225*c*	7.417*c*	2.291*d*
10 µmol dm^{-3} selenite	47.11*bc*	3.994*a*	4.684*b*	6.851*c*	2.183*d*
20 µmol dm^{-3} selenite	40.10*b*	4.013*a*	4.706*b*	6.929*c*	2.014*c*
50 µmol dm^{-3} selenite	38.39*ab*	4.574*b*	3.757*a*	6.929*c*	2.005*c*
100 µmol dm^{-3} selenite	33.44*a*	4.206*a*	3.801*a*	5.285*a*	1.605*b*
10 µmol dm^{-3} selenate	54.86*c*	3.893*a*	4.706*b*	6.309*bc*	2.187*cd*
20 µmol dm^{-3} selenate	50.27*c*	3.880*a*	4.794*b*	6.535*bc*	1.474*ab*
50 µmol dm^{-3} selenate	47.11*bc*	3.924*a*	4.505*b*	5.730*b*	1.421*ab*
100 µmol dm^{-3} selenate	41.91*b*	4.526*b*	4.122*a*	5.772*b*	1.335*a*

* Means within one column marked with the same letters do not differ significantly at $a \leq 0.05$.

Table 2

Concentrations of macronutrients in the shoots of pea seedlings supplied with selenium as selenite and selenate

Treatment	K	P	Ca	S	Mg
	(mg g^{-1} DW)				
Control	42.88d*	8.272*c*	7.432*c*	3.253*b*	2.111*c*
10 µmol dm^{-3} selenite	31.44*c*	7.482*bc*	5.129*b*	2.752*a*	1.846*b*
20 µmol dm^{-3} selenite	25.09*b*	6.310*ab*	5.265*b*	2.791*a*	1.827*b*
50 µmol dm^{-3} selenite	20.19*a*	5.743*a*	3.747*a*	2.673*a*	1.382*a*
100 µmol dm^{-3} selenite	19.99*a*	5.459*a*	4.208*ab*	2.761*a*	1.384*a*
10 µmol dm^{-3} selenate	41.95*d*	7.900*bc*	8.294*d*	6.015*c*	1.843*b*
20 µmol dm^{-3} selenate	73.57*f*	7.409*b*	8.798*d*	8.076*d*	1.868*b*
50 µmol dm^{-3} selenate	58.03*e*	6.962*b*	7.158*c*	7.616*d*	1.608*ab*
100 µmol dm^{-3} selenate	44.76*d*	7.289*b*	7.195*c*	7.088*d*	1.690*ab*

* Means within one column marked with the same letters do not differ significantly at $a \leq 0.05$.

20 µmol dm^{-3} concentration of Se. A similar decreasing tendency in the concentrations of K, Ca and Mg was observed by HAWRYLAK-NOWAK et al. (2015) in shoots of cucumber treated with selenite but not in ones treated with selenate. Regardless of the form, an Se treatment does not have a significant effect on the P concentration in roots of pea seedlings (Table 1). However, the concentration of P shows a significant decrease at 20-100 µmol Se dm^{-3} concentrations in shoots treated both with selenite and selenate (Table 2). HAWRYLAK-NOWAK et al. (2015) also observed a decrease in the P concentra-

tion in cucumber shoots under the influence of selenite at a 30-60 µmol dm^{-3} concentration. In roots of pea seedlings, a significant decrease in the S concentration was observed at a higher Se concentration: 100 µmol dm^{-3} for selenite, and 50 and 100 µmol dm^{-3} for selenate. While in the shoots of selenite-treated pea seedlings S concentration decreased at 10 µmol-Se dm^{-3} concentration and it remained at the constant level at higher concentrations. Many authors observed that Se at a toxic concentration, both as selenite and selenate, caused enhanced lipid peroxidation and damage of plasma membranes, which were determined on the basis of the leakage of ions from the tissue (HARTIKAINEN et al. 2000, CARTES et al. 2005, MORA et al. 2008, FILEK et al. 2010, AGGARWAL et al. 2011, MROCZEK-ZDYRSKA, WÓJCIK 2012). The decrease of macronutrient concentrations found in pea roots and shoots exposed to selenite and selenate may be partly attributable to the damage of plasma membranes.

In contrast to the changes described above, an increase in K, Ca and S concentrations occurred in shoots of plants treated with selenate (Table 2). A stronger effect of selenate on the accumulation of K was observed at the 20 µmol Se dm^{-3} concentration (71.57% above control) than at 50 µmol Se dm^{-3} (35.33% higher than in control). The results of many reports confirm that exposition of plants to Se at toxic concentrations results in tissue dehydration (MOLNÁROVÁ, FARGAŠOVÁ 2009, AGGARWAL et al. 2011, HEGEDÜSOVA et al. 2012, KOSTOPOULOU et al. 2010, MOLNÁROVÁ, FARGAŠOVÁ 2016). Less osmotic uptake of water is the result of lowered concentrations of mineral nutrients. Maintaining osmotic homeostasis requires an increase of osmosis in cells. For this reason, the enhanced uptake and translocation of K to shoots in pea seedlings treated with selenate could have been an element of the mechanism of tolerance towards Se. Our results showed a significant positive correlation between the K concentration in roots and shoots of pea seedlings and the index of tolerance (Figure 4*a*). K is one of the most important inorganic osmotic elements in plants and it can regulate the turgor recovery when its adequate concentration causes the lowering of osmotic potential in plant cells (WANG et al. 2013). KOPSELL et al. (2000) found that the K concentration in cabbage leaves was increasing proportionally to the Se concentration supplied in the selenate form. Moreover, under water deficit conditions, a K concentrations in leaves determines their stomatal conductance (WANG et al. 2013). The Ca concentration was higher by 14.79% compared to control at 10 and 20 µmol Se dm^{-3}. An increased Ca concentration was also observed by HAWRYLAK-NOWAK (2008) in maize shoots under the influence of selenate at 40 µmol dm^{-3} and by DA SILVA et al. (2018) in lettuce plants treated with both selenate and selenite at 10-40 µmol dm^{-3}. Different stresses can elicit in plants the responses which involve long-distance signaling, i.e. information about locally occurring stress is transmitted to distal tissues or organs and results in systemic acquired acclimation. Ca is one of direct mobile systemic signals in the xylem (CHOI et al. 2016). We suggest that the increase in the concentration of this macronutrient in the shoots of selenate-treated pea

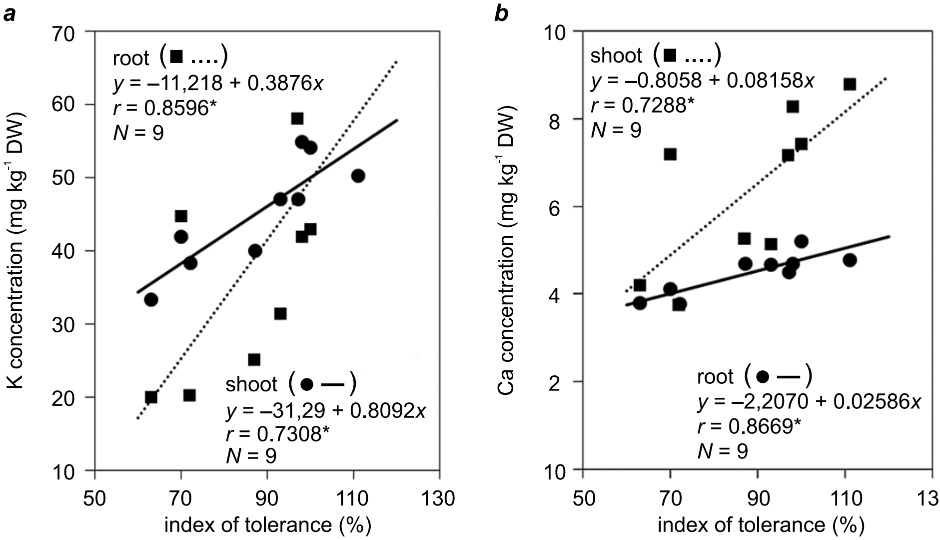

Fig. 4. Correlations between the index of tolerance and K (*a*) or Ca (*b*) concentrations in shoots and roots of pea supplied with selenium as selenite and selenate

seedlings was associated with its signaling role. Regression analysis demonstrated a significantly positive correlation between the Ca concentration in pea seedlings and tolerance index (Figure 4*b*). In pea seedlings' shoots, the highest increase was observed in the S concentration, i.e. 84.90% at 10 μmol Se dm^{-3} and on average 133.42% compared to the control at 20, 50 and 100 μmol Se dm^{-3} (Table 2). Due to the chemical similarity of Se and S, these two elements compete with each other. This competitive effect manifesting in the uptake and translocation of sulphate and selenate has been widely studied and it is well documented that selenate is taken up in plant roots actively by sulphate transporters present in root plasma membrane (Terry et al. 2000, Cartes et al. 2005, Sors et al. 2005). Results of our experiment showed that a greater part of Se supplied in the form of selenate remained in roots of pea seedlings (Figure 1). The main reason was probably the greater affinity of sulphate than selenate to transporters, as evidenced by the high concentration of sulphur in the shoots of pea seedlings treated with selenate (Table 1*b*). Many of sulphate transporters are regulated by the nutritional status of individual tissues to optimize sulphate movement within and between root and shoot (Buchner et al. 2004).

CONCLUSIONS

1. Pea seedlings absorbed Se from nutrient solution and accumulated it mainly in roots. More Se was accumulated in seedlings when it was applied in the form of selenate than selenite.

2. Selenite at a concentration above 20 μmol dm^{-3} significantly limited root elongation. The inhibitory effect of selenate was observed only at 100 μmol dm^{-3}.

3. In general, the treatment of pea seedlings with Se reduced the accumulation of K, Ca, S and Mg in roots and shoots, and P only in shoots. The exception was the shoots of selenate-treated seedlings, in which the concentrations of K, Ca and S increased.

4. Pea seedlings manifested more tolerance towards selenate than selenite. Tolerance of pea seedlings was positively correlated with the K and Ca concentrations.

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