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THE USE OF BIOFORTIFICATION FOR PRODUCTION OF SELENIUM ENRICHED GARDEN PEA

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ABSTRACT

Biofortification of crops with selenium is one of the possible manners on how to increase selenium intake by humans. The effect of selenium fertilization in relation to selenium enrichment of pea and following the phytotoxicity symptoms in garden pea plants was studied. Pot experiments were established with a control variant without selenium addition and four variants where selenium was applied as sodium selenate into the soil in four different concentrations (1 – 6 mg Se.kg^{-1}) before seeding. Garden pea was grown in pots for 60 days and then plant material was dried and submitted to analysis. The total content of selenium was determined by the ZET-AAS method in the roots, above-ground parts of the plant (stems, leaves, extracted pods), and in seeds of a pea. Dean-Dixon´s test and paired t-test (α = 0.05) were used for statistical evaluation of the results. Transfer factors were calculated as a ratio between selenium content (mg.kg^{-1}) in individual plant material and soil. Transfer indexes were calculated as a ratio between selenium content (mg.kg^{-1}) in seeds and roots. The results showed that with the increasing addition of the Se to the soil, its contents in all parts of the plant proportionally increased. The content of the Se increased in the roots 43 to 173-fold, in the above-ground parts 79 to 372-fold, and in the seeds Se was accumulated 130 to 415 times more compared to control. Transfer factors and transport indexes were expressed. Transfer factors for pea varied from 11.05 to 19.25 in the case of Se transfer to the whole pea biomass. In the case of the Se transfer from soil to pea seeds, the highest transfer showed variant with addition 1 mg Se.kg^{-1} and the transfer factor gradually decreased with increasing addition of Se. Based on the amount of biomass produced, the experiments statistically confirmed the phytotoxicity of higher doses (4 and 6 mg Se.kg^{-1}) of selenium to plants. The highest transport index values are shown variants with the Se addition 1 and 2 mg Se.kg^{-1} (2.03 and 1.77, respectively). In these variants, Se was used the most efficiently. Our results showed that the best biofortification results were obtained in experimental variants with the lower selenium additions (1 and 2 mg Se.kg^{-1}).

Keywords: biofortification; selenium; garden pea

INTRODUCTION

Selenium (Se) is an essential micronutrient, which is involved in selenoaminoacids and selenoproteins. It has been proved to have multiple roles in the growth and functioning of living cells and has many crucial biological functions in animals and humans **(Birringer, Pilawa and Flohé, 2002; Tapiero, Townsend and Tew, 2003)**. As a cofactor of the enzyme glutathione peroxidase, and a catalyser of the reduction of peroxides, plays Se role in antioxidant defence. However Se is biologically active at low concentrations for normal growth and development, and at moderate concentrations for homeostatic function, at high concentrations, Se can cause toxicity **(Puccinelli, Malorgio and Pezzarossa, 2017)**. The proposed Recommended Dietary Allowance (RDA) of 55 µg Se day^{-1} for adults and a tolerable upper intake of 400 µg Se day^{-1} has been set by The Food and Nutrition Board of the Institute of Medicine (USA) **(Krinsky et al., 2000)**.

Central Europe has been identified as a region with suboptimal concentrations of selenium in agricultural soils that reflects in low selenium levels in the agricultural products coming from the area **(Sager, 2006)**.

In the past, great efforts have been focused only on increasing crop yields, but enhancing the concentrations of mineral micronutrients including selenium has become an urgent task. Biofortification of trace elements can be achieved by their application within the agronomic process such as soil or foliar fertilization or crop breeding **(El-Ramady et al., 2014)**. Agronomic biofortification with Se could be a powerful tool to remedy Se deficiency as it may increase the Se concentration in the plant sources use in food production **(Poblaciones, Rodrigo and Santamaría et al., 2015)**. Use of common fertilizers with selenium (Se) for crop production is considered as an effective way to produce selenium-rich food and feed.

Legumes and cereals present the basis of the diet of billions of people. Legumes are able to accumulate micronutrients that create the potential to be used in Se biofortification programs **(Thavarajah, Ruszkowski and Vandenberg, 2008; Thavarajah, Warkentin and Vandenberg, 2010)**. **Poblaciones, Rodrigo and Santamaria (2013)** confirmed that the pea has a strong ability to uptake and accumulate Se; therefore, it could be considered as a very strong candidate for inclusion in biofortification programs aiming to increase Se in the food chain. The value of green pea seeds and forages as an alternative protein source can be improved by using agronomic biofortification **(Garousi et al., 2017)**. Biological changes of garden pea (*Pisum sativum* L.) in dependence with the application of inorganic form of Se (and sodium selenate) at different concentrations have been studied. As a plant material was garden pea (*Pisum sativum* L.) that has been recognized as an important source of vitamins C, E, and a group of B vitamins including folic acid derivatives, so-called folates and mineral substances (Fe, K, P, Mn, Mg, Ca). Peas is also rich for soluble and insoluble fiber, vegetable protein and is a source of the macro elements **(Chrenková et al., 2003)**. It has highly valuable in proteins that are similar to those of animal origin. Also, the biological value of the pea protein is higher than that of the cereals. Both pea seeds and forages are rich in protein including lysine and other essential amino acids **(Garousi et al., 2017)**.

Our purpose was to investigate the most efficient selenium additions into the soil to achieve enhancement of Se levels in pea plants as well as identification of potential phytotoxic effects on the plants caused by different levels of inorganic selenium applied.

Scientific hypothesis

Increasing selenium additions in soil reflect in increasing selenium content in pea plants. Higher selenium additions have a potential phytotoxic effect on pea plants.

MATERIAL AND METHODOLOGY

Pot experiments with garden pea were carried out on soil biofortification by sodium selenate. As an experimental material was selected the garden pea (*Pisum sativum* L.) – the Oskar variety (Semo, The Czech Republic). This variety matures relatively early and is suitable for direct consumption and industrial processing, as well. In Slovakia, it is the variety produced intensively in agricultural conditions.

Pot experiments in the phytochamber were based on five variants and eight repetitions. Sodium selenate was applied to the soil substrate 5 days before the planting of pea seeds, in quantities 0, 1, 2, 4, and 6 mg $Se.kg^{-1}$ soil. On the fifth day, we planted 25 seeds of the pea in each container and placed them in the phytochamber with controlled temperature and light regimen. In the stage of technological maturity (60 days after the inception of the experiment) were the plants harvested and the plant material was separated into the total above-ground part, seeds and roots. A soil sample from each pot was taken for analysis and the separated plant material was weighed for each variant for the conversion of the Se content to the total biomass of peas.

The content of the Se has been determined in the roots, above-ground part of the plant (stems, leaves, extracted pods) and grain of peas. Dried soil and plant samples were wet decomposed in the mixture of nitric acid and hydrogen peroxide and after dilution measured according to the previously optimized method described by **Hegedűs et al. (2008)**. The determination of the total selenium was done by atomic absorption spectrometry with Zeeman background correction (ZET-AAS). The apparatus used for analysis was AA240Z Varian (Mulgrave Virginia, Australia), selenium hollow cathode lamp was used for the measurement, and the wavelength was set at 196 nm.

Statistical analysis

Dean-Dixon´s test and paired *t*-test (p <0.05, n = 8) were used for statistical evaluation of the results by a Tanagra 2.0 software.

RESULTS AND DISCUSSION

Pot experiments in the phytochamber were realized to monitor the transfer of Se to the pea plant in model conditions. The results showed that with increasing addition of the Se to the soil, its contents in all parts of the plant proportionally increased (Table 1). Variants with an addition of 1 mg $Se.kg^{-1}$ and 2 mg $Se.kg^{-1}$, showed that the content of the Se was highest in grain, variants with 4 mg $Se.kg^{-1}$ and 6 mg Se.kg kg^{-1} had the highest content of the Se at the root. This fact can be probably linked to the slowdown of plant development due to the phytotoxicity of Se.

Further noted differences between the growth of the individual variants were in the total weight of the plant material where variants with 4 mg $Se.kg^{-1}$ and 6 mg $Se.kg^{-1}$ quantity of plant material was compared to the control 4.5 and 5.7 times lower. Also, the plants of the variants mentioned had at the time of harvesting the less developed seeds compared to the control and variants with the addition of 1 mg $Se.kg^{-1}$ and 2 mg $Se.kg^{-1}$. The content of the Se increased in all parts of the pea plant in variants I to IV, namely at the root of 43 to 173-fold, in the above-ground parts of 79 to 372-fold and in the grain Se was accumulated 130 to 415 times more compared to control. The analysis of the pea showed that selenium fertilization of soil with different selenium additions in the sodium selenate form resulted in a proportional increase of the total selenium content in all parts of the pea.

The statistical evaluation compared the means of the Se content within the different variants in the roots and in the grains. A statistically significant difference (p <0.05) was not found only in the roots between variant I and II and between variants III and IV. For Variant IV, the roots showed symptoms of phytotoxicity and the roots were not able to uptake higher quantities of Se. The statistical evaluation of the Se content in a grain showed a significant difference (p <0.05) between all the variants. The positive correlation between the transfer of Se content from the Se-fortified soil to the seeds of the garden pea reflects the graph of polynomic dependency in Figure 1.

We have found a tight positive correlation which is confirmed with a high coefficient of correlation (R = 0.9969) which was calculated from the coefficient of determination (R^2 = 0.9939) from the dependence of Se transfer from soil enriched with sodium selenate to garden pea seeds. With regard to doses applied, there was found also a highly tight relationship between the Se content in other plant parts and the Se applied.

The positive effect of sodium selenate fertilization on enhancement of selenium content in peas confirmed also authors **Poblaciones, Rodrigo and Santamaria (2013), Poblaciones, Rodrigo and Santamaria (2015), Garousi et al. (2017), Hegedűsová et al. (2015), Smrkolj et al (2006)** and **Hegedűs, Hegedűsová and Šimková (2007)**.

Our experiments in phytochamber pointed at several times the higher accumulation of selenium in the conditions of regulated light regime compared to the experiments in greenhouse or field conditions. **Ros et al. (2016)** explored available results from selenium fertilization experiments in different conditions and across all observations, the strongest accumulation was achieved in the experiments in growth chambers, mainly in solutions. Probably this positive effect could be explained by even larger volatilization and transfer between the high Se-treatments to control and low Se-treatments during the growth.

Pot experiments with peas were realized with the same variants of the Se addition in outdoor conditions with normal light conditions, so the impact of UV-B should also be taken into account from the phytotoxicity point of view when comparing the results of experiments in the phytochamber and in the outer environment. UV-B radiation in normal light conditions for the accumulation of Se and the signs of phytotoxicity of the Se on the pea.

The Se phytotoxicity symptoms include the amount of biomass produced. The experiments carried out confirmed statistically the phytotoxicity of higher selenium doses (4 and 6 mg Se.kg^{-1}) to plants, based on the weight of the biomass produced is given in Table 2.

From the evaluation of the biomass weights, it can be assumed that the first signs of phytotoxicity begin to emerge after the application of 2 mg Se.kg^{-1}. The symptom was later maturation of the seeds compared to the control or variant I. The significant braking effect on the formation of biomass and seeds had Se additions 4 and 6 mg Se.kg^{-1}. The limit concentration with the phytotoxic effect on garden pea will probably be a selenium addition of 3 mg.kg^{-1}, which also confirmed the results of the work of **Vargová et al. (2009).**

In Figure 2, the phytotoxic effect of the Se addition in variants III and IV (4 and 6 mg Se.kg^{-1}) can be clearly observed. It was visible from the first days of plant germination and persisted throughout growing and ripening until the plants were harvested.

Table 1 Selenium content in pea and soil substrate - results of sodium selenate fertilization in the phytochamber.

Variant	**Plant part**	**Dry matter**	**Se content in dry matter**	**Total Se content in the whole phytomass**	**Se content in soil after experiment**
Se (mg.kg^{-1} substrate)		(%)	mean ±*SD* (mg.kg^{-1})	(mg/pot)	mean ±*SD* (mg.kg^{-1})
K 0 mg Se	root	11.82	1.90 ±0.37	0.007	0.26 ±0.06
	above-ground part	19.55	0.56 ±0.24		
	seed	22.29	0.74 ±1.96		
I 1 mg Se	root	16.40	81.54 ±35.44*	1.204	1.32 ±0.47
	above-ground part	32.64	65.56 ±20.58*		
	seed	27.96	96.50 ±39.72*		
II 2 mg Se	root	14.57	121.34 ±45.79*	1.171	2.12 ±1.03
	above-ground part	21.21	108.32 ±7.99*		
	seed	19.92	137.68 ±21.74*		
III 4 mg Se	root	15.27	303.16 ±94.79*	1.633	3.18 ±0.48
	above-ground part	24.31	245.70 ±66.58*		
	seed	20.02	242.20 ±31.86*		
IV 6 mg Se	root	34.85	327.66 ±43.26*	1.587	4.84 ±1.34
	above-ground part	22.13	308.80 ±74.01*		
	seed	18.05	309.00 ±65.32*		

Note: *statistically significant difference (comparison with the control variant; p <0.05, t_{krit} = 2.365).

Table 2 Total weight of the biomass produced for garden pea.

Plant part	**Weight of fresh biomass** (g/pot) K	I	II	III	IV
	mean ±*SD*	**mean ±*SD***	**mean ±*SD***	**mean ±*SD***	**mean ±*SD***
root	17.18 ±2.28	19.85 ±3.59	18.19 ±4.59	7.41 ±2.33*	8.46 ±1.58*
above-ground part	36.14 ±5.62	37.75 ±7.13	32.88 ±3.83	20.27 ±1.77*	9.99 ±5.03*
seed	5.93 ±2.07	4.95 ±2.02	3.45 ±1.30*	1.48 ±0.26*	0.17±0.06*

Note: *statistically significant difference (comparison with the control variant; p <0.05, t_{krit} = 2.365). Experimental variants: K = 0; I = 1; II = 2; III = 4; IV = 6 mg Se.kg^{-1} substrate.

The selenium supplement in these variants caused by braking growth, later development of flowers, and delayed seed formation.

The flowering in variant III occurred approximately 4 days and in variant IV by 7 days later compared to the control variant K. The results indicated that the phytotoxicity of the salt applied was not observed in the germination but later at the stage of growth, probably when the young plant starts to behave autotrophic and received dissolved ions of selenate from the soil solution. The Se concentration at which the phytotoxicity begins to occur is different for each species of cultural plant. Determination of the concentration limit of Se phytotoxicity is dependent on the soil enrichment technology. In recent years, increased attention has been paid to the protective role of Se in the plant against the stress caused by the higher intensity of UV radiation. **Hartikainen and Xue (1999), Germ, Kreft and Osvald (2005)** and **Smrkolj et al. (2005)** described that appropriate levels of Se in plants help to protect against oxidative stress caused by increasing UV-B radiation. The results confirm that in plants under normal light conditions, the appropriate addition of selenium helps to produce a larger crop.

The activity of the antioxidant enzymes superoxide dismutase, guaiacol peroxidase, and glutathione reductase in cucumber leaves was studied by **Jain, Kataria and Guruprasad (2004).** They found that the activity of antioxidant enzymes increased in cucumber leaves as a

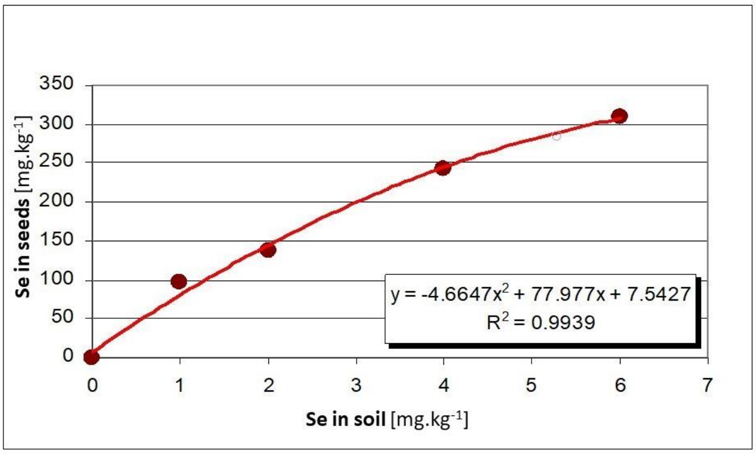

Figure 1 Dependence of Se transfer from soil enriched with sodium selenate to garden pea seeds.

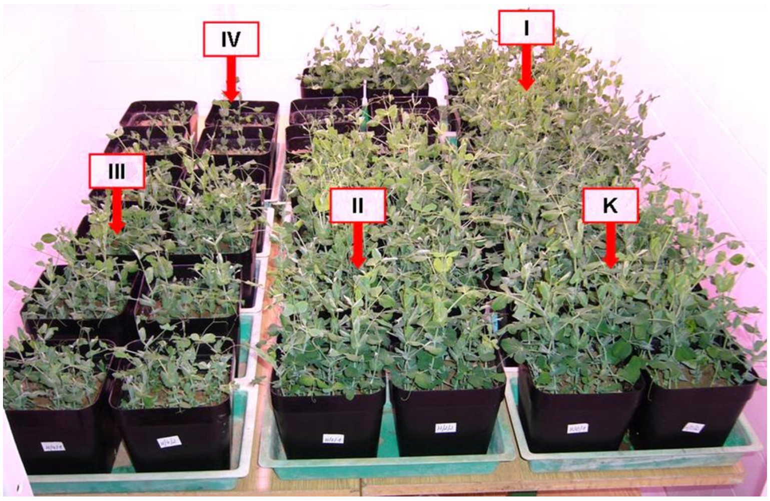

Figure 2 Visual comparison of individual experimental variants in phytochamber 20 days after experiment. establishment

Table 3 Selenium transfer factors and transport indices in garden pea.

Variant	Se addition (mg.kg^{-1})	Mean content of Se in fresh biomass (mg.kg^{-1})	TF_{SP}	TF_{SG}	TI_{RG}
K	0	0.17	0.00	0.00	0.79
I	1	19.25	19.25	25.03	2.03
II	2	22.09	11.05	13.76	1.77
III	4	57.20	14.30	12.19	0.98
IV	6	83.53	13.92	9.30	0.50

Note: $\mathbf{TF_{SP}}$ – transfer factor soil – plant, $\mathbf{TF_{SG}}$ – transfer factor soil – seeds, $\mathbf{TI_{RG}}$ – transport index root – seed.

response to the increasing intensity of UV-B radiation. Free radicals generated by UV-B radiation are likely to be associated with the induction of a defensive antioxidant system. The activity of UV-B induced enzymes is altered by the external application of antioxidants, which are also capable of scavenging free radicals and relieving plant stress due to UV-B radiation.

These facts lead to an explanation of the differences observed between the light regime during growth in the phytochamber (lower intensity of UV radiation) and normal outdoor light conditions. Increased cumulation of Se in peas in all variants compared to those grown under normal light conditions is evidence of this.

Transfer factor and transport index

The transfer factor (TF) is considered as an indicator of the element's entry into the plant. The concentration of chemical elements in soil and dry matter of plant samples are used for calculation **(Uchida, Tagami and Hirai, 2007; Blanco Rodríguez et al., 2006; Bitterli, Banuelos and Schulin, 2010)**. Transfer factors for peas varied from 11.05 to 19.25 in the case of Se transfer to the whole pea biomass (TF_{SP}), in the case of the Se transfer from soil to pea seeds (TF_{SG}), the highest transfer showed in variant I and gradually decreased with increasing addition of Se (Table 3). In general, transfer factors are higher than those reported in the literature. Literary data describe transfer factors under natural conditions without soil fortification, therefore selenium content in vegetables was naturally lower.

The transfer factor (TF) depends on the type of plant, part of the plant, and the soil characteristics as well as the chemical forms of the element in the soil **(Bitterli, Banuelos and Schulin, 2010)**. The leaves of plants have a higher transpiration rate, therefore they require more water and essential elements than other parts of the plant, and therefore trace elements are used to accumulate to a higher extent in the leaves. Therefore, there is a higher transfer factor in these parts of the plant compared to the others, as confirmed by several authors, **Uchida Tagami and Hirai (2007)** and **Blanco Rodriguéz et al. (2006)**.

Uchida Tagami and Hirai, (2007) reported TF in for 68 vegetable and cereal samples. At a mean Se content in the soil of 0.54 $mg.kg^{-1}$, cabbage had a TF of 0.011 – 0.036, soybean 0.016 and lettuce 0.017 – 0.0083.

A transport index (TI) is used to evaluate the ability of a plant to transport an element to a collectible portion of a plant. **Blanco Rodriguéz et al. (2006)** defines the transport index as the ratio of element concentration in above-ground resp. in the consumption part and its concentration in the root. The transport index was used to quantify the relationship of Se transportation from roots to grains. The Se concentration ratio in the grain to the concentration in the root in peas is expressed by the TI_{RG} values in Table 3. The highest TI_{RG} values are shown for variants I (2.03) and II (1.77). These variants used Se the most efficiently.

CONCLUSION

Our findings proved that Se biofortification has positive effects on the improvement of selenium status in garden pea that can provide Se enriched food source for the production of a proper functional product. The results suggest that peas are suitable vegetable species for agronomic biofortification and can introduce Se into the human and animal diet. Garden pea has a great ability to accumulate high amount of Se in the grain. Our investigations proved that a relatively small amount of Se fertilizer can highly enhance Se supply in the grain. A strong linear relationship between the total Se content in pea and the dose rates of the application were also proved, however from the phytotoxic point of view, concentrations over 2 mg Se kg^{-1} of soil resulted in a decrease of plant growth and delay of seed development. This fact presents valuable information for potential recommendations for agronomic practice. In conclusion, our results could be used as valuable information for suitability of peas for inclusion in Se biofortification programs; however, another additional research on the sustainability of biofortification with even low selenate addition into different soils should be considered in relation with a potential environmental impact on arable soils and surface waters, as well.

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