ADDITION OF Se AFFECTED CONCENTRATION OF Se IN THE SECOND GENERATION OF TARTARY BUCKWHEAT PLANTS

DODATEK SELENA JE VPLIVAL NA KONCENTRACIJO Se V POTOMKAH S Se OBRAVNAVANIH RASTLIN

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ABSTRACT

Addition of Se affected concentration of Se in the second generation of Tartary buckwheat plants

Tartary buckwheat and common buckwheat plants were grown on the field in natural conditions at high elevation. Tartary buckwheat plants were foliarly treated with Se and S (126 µM for each element). Seeds were collected and sown to obtain the progeny of Se-and S- treated plants. Concentration of Se was measured in these plants. Concentration of Se in all plant parts was similar in control and S treated plants. The concentration of Se was the highest in plants from seeds grown on Se treated plants in roots and leaves. It is shown that Se treatments in previous generation affected Se concentration in the progeny of Tartary buckwheat. Results also showed that in untreated plants, Se concentration was higher in Tartary comparing to common buckwheat in roots and seeds.

Key words: Tartary buckwheat, selenium, sulphur

IZVLEČEK

Dodatek selena je vplival na koncentracijo Se v potomkah s Se obravnavanih rastlin

Navadna in tatarska ajda sta uspevali na njivi na visoki nadmorski višini. Tatarsko ajdo smo listno škropili s selenom in žveplom (126 µM za vsak element). Na koncu rastne sezone smo na rastlinah zbrali semena in jih naslednje leto posejali. Na ta način smo dobili potomce s Se in S obravnavanih rastlin. V teh rastlinah smo merili koncentracijo Se. Koncentracija Se pri tatarski ajdi je bila v vseh rastlinskih delih podobna pri kontrolnih rastlinah in rastlinah, zrastlih iz semen, obravanavanih s S. Najvišjo koncentracijo Se v listih in koreninah so imele rastline, zrasle iz semen, nabranih na rastlinah, listno škropljenih s Se. Rezultati kažejo, da se obravnavanje s Se v prvi generaciji izrazi v večji koncentraciji Se v potomkah teh rastlin pri tatarski ajdi. Izsledki raziskav so prav tako pokazali, da je koncentracija Se v kontrolnih rastlinah v koreninah in semenih višja pri tatarski ajdi v primerjavi z navadno ajdo.

Ključne besede: tatarska ajda, selen, žveplo

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1 INTRODUCTION

Tartary buckwheat (*Fagopyrum tataricum* Gaertn.) is primarily grown in mountainous areas and plateaus in regions of Asia (for example, the mountainous areas of the Western part of Sichuan in China, and Nepal), as well as in a few areas of Europe (Bonafaccia et al. 2003, Fabjan et al. 2003). Germ (2004) reported that enhanced UV-B radiation induced the synthesis of UV absorbing compounds in common buckwheat. Of the main importance in Tartary buckwheat are flavonoids (rutin and quercetin) and amino acids (arginine, lysine, threonine), proteins and trace elements, for example magnesium, potassium, zinc, selenium, etc. (Yang 2014). Tartary buckwheat contains about hundred times more rutin compared to common buckwheat (Fabjan et al. 2003). Leaves of Tartary buckwheat are used for preparing tea, the fresh sprouts are used as a vegetable, and from milled grain flour is produced. Tartary buckwheat plants are free of gluten and safe for patients with celiac disease (Bonafaccia et al. 2003). Selenium (Se) is a trace element, essential nutrient for humans (Yu & Gu 2008) and is predominantly provided from cereals, meat and fish (Combs 2001). Lampis et al. (2009) stated that the boundary among essentiality, deficiency, and toxicity of Se is narrow and mainly depends on the chemical forms and concentrations. Several studies have suggested that selenium supplementation can reduce the risk of some cancers (Carey et al. 2012). Selenium has not been classified as an essential element for plants, although its role has been considered as beneficial in plants capable of absorbing large amount of the element (Germ et al. 2005, Djanaguiraman et al. 2010, Malik et al. 2012). Se is together with Al, Co, Na and V included to the group of "beneficial element" (Kopsell & Kopsell 2007). Up to 1 billion people worldwide are selenium-deficient due to low Se concentrations and its availability in soils and consequently low concentrations in crop plants (White & Broadley 2009). Selenium deficiency leads to health disorders like heart disease, hypothyroidism and general impairment of the immune system (Ellis & Salt 2003). Due to chemical similarities between Se and S (White et al. 2004), the uptake, transport and assimilation of selenate follow the sulfate pathway (Sors et al. 2005). Selenate is thought to enter root cells through sulphate transporters in the plasma membrane (White et al. 2004). Higher concentrations of absorbed Se can be harmful due to non-specific replacement of S by Se in proteins and other sulfur compounds (Terry et al. 2000) and due to enhanced production of reactive oxygen species. White et al. (2004) evidenced that Se toxicity was directly related to the ratio between Se and S in the shoots. This indicates that Se toxicity occurs because Se and S compete for a biochemical process, such as assimilation into the amino acids of essential proteins. Angiosperms have similar or lower shoot Se/S quotients than those of the rhizosphere solution (White et al. 2004, Galeas et al. 2007). There is no information available regarding possibly antagonistic effect of Se and S on concentration of Se in different plant organs in progeny of Se and S treated plants.

The aim of our work was to determine the possible impact of Se and S treatment on Se concentration in progeny of Tartary buckwheat plants.

2 MATERIALS AND METHODS

Field experiment

Plants of Tartary buckwheat and common buckwheat were sown in acidic soil in the field in Bosnia. Tartary buckwheat was foliarly treated with Se and S. In the experiment 10 grams seeds per m-2 were sown on randomly distributed plots 1 m x 3 m (S treated, Se treated, control), in three blocks. Sowing date was May 24 in village Donje Selo, near Ilijaš, which is located at elevation 1004 m. Foliar spraying with sodium selenate (126 µM) respectively the same molarity of sodium sulphate solution was performed before the beginning of flowering, on June 27. The average temperature during the experiment was 14 ^{o}C and amount of precipitation was 411 mm. Seeds from plants of both species were collected and sown to the soil next year. Concentration of Se was measured in progeny of treated Tartary buckwheat plants in roots, leaves and seeds.

The total Se content in roots, leaves and seeds was determined by hydride generation atomic fluorescence spectrometry (HG-AFS). Method of digestion and optimal measurement conditions were described in detail by Smrkolj and Stibilj (2004). The accuracy of the method was checked with the certified reference material Spinach Leaves (NIST 1570a).

For statistical analysis SPSS Statistic software, version 20,0 (IBM) was used. The normal distribution of the data was tested with Shapiro-Wilk test. Differences between control and treated plants were evaluated by ANOVA followed by Tukey's post-hoc multiple comparison tests. Differences at $p < 0.05$ were considered statistically significant.

3 RESULTS AND DISCUSSION

The concentration of Se in plants from seeds, grown on S treated plants did not differ from control (Table 1).

Table 1: Concentration of Se (ng/g dry matter) in progeny of control, Se and S treated Tartary buckwheat

	Roots		Leaves		Seeds	
Treatments	Average	SD	Average	SD	Average	SD
Control	26.3a	6.4	16.7a	5.3	20.9a	5.5
Sulphur	20.7a	2.8	20.6a	1.8	16.7a	5
Selenium	34.4b	3.5	25.6b	5.3	13.8a	2.1

Means (calculated from 4 independent measurements) not sharing the same letter, a and b, differ significantly ($p < 0.05$) within each column.

Se was effectively assimilated by the plants and taken into the seeds and in plants, grown from these seeds, the concentration of Se was higher than concentrations of Se in control plants in roots and leaves. Similar outcomes have been also given in the study from Kreft et al. (2013). In this research as in the present, the leaves were sprayed with 10 mg Se(VI) L^{-1} at the beginning of flowering. Epigenetic effect of Se was reported also in the case of pea (Smrkolj et al. 2006 a), where the transfer of Se from Se-enriched seeds to young plants has been established. Plant species differ in their abilities to accumulate Se (Dhillon & Dhillon 2003). Golob et al. (2015) stated that foliar application with 0.05 mg Se L^{-1} in the form of selenate as soil and foliar application increased Se concentration of Tartary buckwheat grain by 4 fold. In addition, Tartary buckwheat foliarly sprayed with the solution of 20 mg Se L^{-1} in the form of selenite increases Se concentration in seeds by 276 fold (Golob et al. 2016). Increased Se concentrations in Tartary buckwheat were known also for foliarly treated plants in the experiments from Smrkolj et al. (2006 b).

Foliarly added Se was translocated to different plants parts in Tartary buckwheat (Table 2).

Table 2: Concentration of Se (ng/g dry matter) in progeny of untreated Tartary buckwheat and common buckwheat

	Roots		Leaves		Seeds	
Species	Average	SD	Average	SD	Average	SD
Tartary buckwheat	26.3a, A	6.4	16.7a, B	5.3	20.9a, AB	5.5
Common buckwheat	14.4b, A	2.2	21.7a, B	3.5	11.7b, A	2

Means (calculated from 4 independent measurements). Rows not sharing the same upper case letter are significantly different ($p < 0.05$) within each species between plant parts. Columns not sharing the same lower case letter are significantly different ($p < 0.05$) between species within the each plant part.

Concentrations of Se in Tartary buckwheat were higher in roots comparing to leaves. The concentration of Se in plant roots is much higher in comparison to the leaves and seeds also in the study from Kreft et al. (2013). Authors stated that in Tartary buckwheat, the root system is formed early in the plant development. Since Tartary buckwheat is a plant with indeterminate growth, leaves are formed continuously during the plant growth and the seeds are formed at the last stage of plant development. Results from both studies show that the available Se was taken up early in the development of these plants, with little translocated in the later growth stages.

S and Se metabolism in plants are closely interrelated (Terry et al. 2000). Thus it was well worthy to study the possible interactions regarding the absorption ability for Se in Se and S treated plants. Concentration of Se in progeny of Tartary buckwheat was higher in Se treated plants comparing to S treated plants and control in roots and leaves. These results implied that there was no negative effect of S at concentrations 126 µM, on Se accumulation in Tartary buckwheat plants in the next generation. Similarly Gupta and White (1975) reported that the addition of lime, S, B, and Mo to the soil under field conditions did not affect the Se concentration in plant tissues. In opposite, sulphur decrease plant selenium uptake in northern mixed prairie when soil selenium-fertilized and sulphur plus selenium fertilized plants were compared (Milchunas et al. 1983). In addition, Murphy and Quirke (1997) who studied the effects of S fertiliser on the uptake of selenium by herbage found out that the uptake of native and soil-applied Se by herbage was reduced by S fertilisation. Tartary buckwheat had higher amount of Se in roots and seeds comparing to common buckwheat (Table 2). Similar results were published also by Smrkolj et al. (2006b).

4 CONCLUSIONS

Results showed that treatments of maternal plants influenced the concentration of Se in second generation of Tartary buckwheat. An impact of Se from treated Tartary buckwheat plants on the progeny plants was reported previously for pea plants and Tartary buckwheat plants. The observed impact of tested combinations with selenium and sulphur foliar spraying on the Se concentration in Tartary buckwheat suggested no inhibitory effect of S treatment on concentration of Se.

5 POVZETEK

V Sloveniji poznamo dve vrsti ajde: navadno ajdo (*Fagopyrum esculentum* Moench) in tatarsko ajdo (*Fagopyrum tataricum* Gaertn.). Gojeni obliki navadne in tatarske ajde izvirata z visokih pokrajin jugozahodne Kitajske: vzhodnega Tibeta, Junana in Sečuana. Raziskave kažejo na to, da ima tatarska ajda večjo vsebnost antioksidantov in zato večji antioksidativni potencial kot navadna ajda. Tatarska ajda vsebuje do 100 krat več rutina kot navadna ajda. Selen je esencialen element za ljudi in živali, njegova esencialnost za rastline še ni dokazana. Mnoge raziskave so pokazale pozitivne vplive selena tudi pri rastlinah. Selen je v številnih primerih omilil negativne učinke sevanja UV-B: oblažil znižanje biomase rastlin in upočasnil staranje ter povečal vsebnost antioksidantov in aktivnost antioksidantskih encimov ter pozitivno vplival na rastline, podvržene pomanjkanju vode. Navadna in tatarska ajda sta uspevali na polju na visoki nadmorski višini 1004 m v vasi Donje Selo, blizu kraja Ilijaš v Bosni. Tatarsko ajdo smo listno škropili s Se in S. V poskusu smo 10 g semen na m-2 posejali na naključno porazdeljene razdelke 1 m x 3 m (obravnava s Se in obravnava s S ter kontrola) na tri bloke. Sejali smo 24 maja in škropili z Na selenatom 126 µM in s isto molarnostjo Na sulfata pred fazo cvetenja, 27 junija. Semena rastlin obeh vrst smo zbrali in jih naslednje leto posejali na njivo. Pri tatarski ajdi smo merili koncentracijo Se v potomkah rastlin, zrastlih iz semen na rastlinah, obravnavanih s Se in S. Koncentracijo Se smo merili v koreninah, listih in semenih. Koncentracija Se pri tatarski ajdi je bila v vseh rastlinskih delih podobna pri kontrolnih rastlinah in rastlinah, zrastlih iz semen, obravanavanih s S. Najvišjo koncentracijo Se v koreninah in listih so imele rastline, zrasle iz semen, nabranih na rastlinah, listno škropljenih s Se. Rezultati kažejo, da obravanavnje s Se v prvi generaciji vpliva na koncentraciji Se v potomkah teh rastlin pri tatarski ajdi. Izsledki raziskave so tudi pokazali, da dodatek S ni deloval zaviralno na privzem Se pri tatarski ajdi. Koncentracija Se pri tatarski ajdi je bila najvišja v koreninah. Podobne rezultate so dobili tudi drugi raziskovalci. Izsledki raziskav so prav tako pokazali, da je koncentracija Se v koreninah in semenih v kontrolnih raslinah višja pri tatarski ajdi v primerjavi z navadno ajdo.

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REFERENCES

Bonafaccia, G., M. Marocchini & I. Kreft, 2003. *Composition and technological properties of the flour and bran from common and tartary buckwheat.* Food Chem. (Amsterdam) 80: 9-15.

Carey, A. M., E. Lombi, E. Donner, M. D. de Jonge, T. Punshon, B. P. Jackson, M. L. Guerinot, H. P. Adam & A. A. Meharg, 2012: *A review of recent developments in the speciation and location of arsenic and selenium in rice grain*. Anal. Bioanal. Chem. (Berlin) 402(10): 3275-3286.

Combs, G. F. 2001: *Selenium in global food systems*. Br. J. Nutr. (Cambridge) 85: 517-547.

Dhillon, K. S. & S. K. Dhillon, 2003: *Distribution and management of seleniferous soils*. Advances Agron. (Amsterdam) 79: 119-184.

Djanaguiraman, M., P. V. V. Prasad & M. Seppanen, 2010. *Selenium protects sorghum leaves from oxidative damage under high temperature stress by enhancing antioxidant defence system*. Plant Physiol. Biochem. (Paris) 48: 999-1007.

Ellis, D. R. & D. E. Salt, 2003: *Plants, selenium and human health*. Curr. Opin. Plant Biol. (Amsterdam) 6: 273-279.

Fabjan, N., J. Rode, I. J. Košir, Z. Wang, Z. Zhang & I. Kreft, 2003: *Tartary buckwheat (Fagopyrum tataricum Gaertn.) as a source of dietary rutin and quercitrin*. J. Agric. Food Chem. (München) 51: 6452-6455.

Galeas, M. L., L. H. Zhang, J. L. Freeman, M. Wegne & E. A. H. Pilon-Smits, 2007: *Seasonal fluctuations of selenium and sulfur accumulation in selenium hyperaccumulators and related nonaccumulators*. New Phytol. (Lancaster) 173: 517-525.

Germ, M., 2004: *Impact of UV-B radiation on plants = Vpliv UV-B sevanja na rastline*. Razprave IV. razreda SAZU (Ljubljana) 45(1): 49-61.

Germ, M., I. Kreft & J. Osvald, 2005: *Influence of UV-B exclusion and selenium treatment on photochemical efficiency of photosystem II, yield and respiratory potential in pumpkins* (*Cucurbita pepo* L.). Plant Physiol. Biochem. (Paris) 43: 445-448.

Golob, A., V. Stibilj, I. Kreft & M. Germ, 2015: *The feasibility of using Tartary buckwheat as a Se–containing food material*. J. Chem. (Cairo) ID 246042, 4.

Golob, A., M. Germ, I. Kreft, I. Zelnik, U. Kristan & V. Stibilj, 2016: *Selenium species and energy management in Se treated buckwheat*. Acta Bot. Croat. (Zagreb) 75(1): 17-24.

Gupta, U. C. & K. A. Winter, 1975: *Selenium content of soils and crops and the effects of lime and sulfur on plant selenium*. Can. J. Soil Sci. (Ottawa) 55: 161-166.

Kreft, I., Š. Mechora, M. Germ & V. Stibilj, 2013: *Impact of selenium on mitochondrial activity in young Tartary buckwheat plants.* Plant Physiol. Biochem. (Paris) 63: 196-199.

Kopsell, D. A. & D. E. Kopsell, 2007: *Selenium*. In: Barker, A.V., D. J. Pilbeam (eds.), *Handbook of Plant Nutrition*. CRC Press, Taylor & Francis Group 515-549. Boca Raton, London, New York

Lampis, S., A. Ferrari, A. C. Cunha-Queda, P. Alvarenga, S. Di Gregorio & G. Vallini, 2009: *Selenite resistant rhizobacteria stimulate SeO_3^{2-} phytoextraction by Brassica juncea in bioaugmented water-filtering artificial beds*. Environ. Sci. Pollut. Res. (Berlin) 16: 663-670.

Malik, J. A., S. Goel, N. Kaur, S. Sharma, I. Singh & H. Nayyar, 2012: *Selenium antagonises the toxic effects of arsenic on mungbean (Phaseolus aureus* Roxb.*) plants by restricting its uptake and enhancing the antioxidative and detoxification mechanisms.* Environ. Exp. Bot. (Amsterdam) 77: 242-248.

Milchunas, D. G., W. K. Lauenroth & J. L. Dodd, 1983: *The interaction of atmospheric and soil sulfur on the sulfur and selenium concentration of range plants*. Plant Soil (Berlin) 72(1): 117-125.

Murphy, M. D. & W. A. Quirke, 1997: *The effect of sulphur/nitrogen/selenium interactions on herbage yield and quality.* Irish J. Agr. Food Res. (Carlow) 36: 31-38.

Smrkolj, P. & V. Stibilj, 2004: *Determination of selenium in vegetables by hydride generation atomic fluorescence spectrometry.* Anal. Chim. Acta (Amsterdam) 512: 11-17.

Smrkolj, P., M. Germ, I. Kreft & V. Stibilj, 2006a: *Respiratory potential and Se compounds in pea* (*Pisum sativum* L.) *plants grown from Se-enriched seeds*. J. Exp. Bot. (Oxford) 57: 3595-3600.

Smrkolj, P., V. Stibilj, I. Kreft & M. Germ. 2006b: *Selenium species in buckwheat cultivated with foliar addition of Se(VI) and various levels of UV-B radiation*. Food Chem. (Amsterdam) 96: 675-681.

Sors, T. G., D. R. Ellis & D. E. Salt, 2005: *Selenium uptake, translocation, assimilation and metabolic fate in plants*. Photosynth. Res. (Berlin) 86(3): 373-389.

Terry, N., A. M. Zayed, M. P. de Souza & A. S. Tarun, 2000: *Selenium in higher plants.* Ann. Rev. Plant Physiol. Plant Mol. Biol. (Berlin) 51: 401-432.

White, P. J., H. C. Bowen, P. Parmaguru, M. Fritz, W. P. Spracklen, R. E. Spiby, M. C. Meacham, A. Mead, M. Harriman, L. J., Trueman, B. M., Smith, B. Thomas & M. R. Broadley, 2004: *Interactions between selenium and sulphur nutrition in Arabidopsis thaliana*. J. Exp. Bot. (Oxford) 55(404): 1927-1937.

White, P. J. & M. R. B Broadley, 2009: *Biofortification of crops with seven mineral elements often lacking in human diets: iron, zinc, copper, calcium, magnesium, selenium and iodine*. New Phytol. (Lancaster) 182: 49-84.

Yu, X. Z. & J. D. Gu, 2008: *Differences in uptake and translocation of selenate and selenite by the weeping willow and hybrid willow.* Environ. Sci. Poll. Res. (Berlin) 15: 499-508.

Yang, J., 2014: *Application perspective of tartary buckwheat as sports supplements*. J. Chem. Pharm. Res. (Jaipur) 6(3): 1239-1241.