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ORIGINAL PAPER

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INCREASING OF SELENIUM CONTENT AND QUALITATIVE PARAMETERS IN GARDEN PEA (*Pisum sativum* L.) AFTER ITS FOLIAR APPLICATION

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ABSTRACT

The presented paper deals with monitoring of the ways of selenium (Se) content increase by foliar biofortification with inorganic Se in two varieties of garden pea *Pisum sativum* L. (Premium, Ambassador) in the conditions of south Slovakia in 2014–2015. The results of experiments show that treatment of plants with two doses of Se concentration (50 g and 100 g Se ha^{-1} in the form of a sodium selenate anhydrous solution) at the flowering stage significantly increased the total Se content in the seeds of both varieties. Following the results by the consumption of 25 g of dried seeds of peas or 100 g of fresh pea seeds after the biofortification with 100 g Se ha^{-1} a recommended daily dose of Se in humans may be covered. The significantly positive influence of Se application on the total polyphenols content (TPC) has been confirmed in the both varieties after application of dosage in 100 g Se ha^{-1} (52% and 33%). A significant increase in the average value of total antioxidant capacity (TAC by DPPH method) in garden pea var. Ambassador was observed after the application of both doses of Se, in case of Premium variety only after application with a 100 g Se ha^{-1}. Significantly increasing level of TAC by PCL (photochemiluminescence) method was found out only in case of var. Premium.

Key words: pea varieties, biofortification, selenization, total polyphenols, antioxidant capacity

INTRODUCTION

Green pea (*Pisum sativum* L.) as a good source of vegetable protein, vitamins, fibre and micronutrients belongs to Pea family – *Fabaceae* [Hegedűsová et al. 2015]. Pea is the fourth most important grain legume crop of the world, as measured by production (441.53×10^3 t) [Shahid et al. 2014, Šlosár et al. 2016]. Biofortification, the process of transferring nutrients into food crops, provides a comparatively cost-effective, sustainable, and long-term means of delivering more micronutrients [Saltzman et al.

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2013]. Selenium (Se) which has antioxidant, anticancer, and antiviral properties, is an essential micronutrient for humans and animals. That's the reason of its repeated using in biofortification programs. In the soil Se is rapidly reduced to insoluble forms and usually less than 10% of the applied Se was taken up by the crop. Another method of transferring Se to plants is foliar application of Se, either as sodium selenate or sodium selenite [Haug et al. 2007]. Very important reason of foliar application preference is possibility of soil contamination, since by long-term using of selenium at higher concentrations in the soil it can accumulate and act phytotoxically. Foliar application was also shown to be several times more efficient than application in fertilizers [Aspila 2005]. On the other hand, there is risk of Se uptake by the crop because of spraying conditions. Application of Se in forms which are for plants the most usable is also very important. In respect of a few studies connected with pea biofortification by Se, sodium selenate was much more effectively taken up by plants compared to sodium selenite [Poblaciones et al. 2013]. Date of application and dose has to be also very carefully considered, because there is very thin line between toxicity and benefits of Se in human diet. Double biofortification of wheat plants more effectively increases the Se concentrations in the grain, in comparison to single biofortification with Se [Germ et al. 2013]. Selenium at low doses has been shown to promote the growth of plants, nevertheless, at high concentrations this metalloid is toxic [Gajewska et al. 2013]. The nationwide supplementation of fertilizers with sodium selenate was shown to be effective and safe in increasing the Se intake of the whole population in Finland [Alfthan et al. 2015], while the author stated that one of the reasons for full-scale fortification was, that the soils in Finland are poor in Se. Similarly so far as the Slovak soils are not rich in Se, it is necessary to increase their content in the agricultural comodities, just at the beginning of food chain [Ducsay et al. 2009]. Increasing the Zn and Se concentrations of food crop plants would result in improved human health, which is particularly important as Zn and Se deficiencies remain a worldwide problem [Carey et al. 2012]. Selenium, folic acid, iodine and vitamin C are the four micronutrients with the highest risk for low diet intake in Europe [Viñas et al. 2011]. Selenium is arguably the naturally occurring trace element of greatest concern worldwide. In excessive amounts it can lead to toxicosis and teratogenesis in animals, while the impact of Se deficiency can be even more significant [Bañuelos et al. 2013]. Selenium bioavailability varies according to the Se source and nutritional status of the subject, being significantly higher for organic forms of Se [Navarro-Alarcon and Cabrera-Vique 2008]. The European Recommended Dietary Allowance (RDA) of Se for humans is about 55 μg Se day^{-1} [Elmadfa 2009]. However, this value does not reflect its different chemical forms [Thiry et al. 2012]. The required Se intake can be obtained from crops produced on selenium-rich soils or by genetic breeding of new suitable accessions that could accumulate more Se in seeds [Lachman et al. 2011]. One of the great aptitudes of peas is ability to accumulate Se in the grain, which shows a great potential to be used as a "functional food" in Se biofortification programs [Poblaciones et al. 2013]. The raw seeds of pea are the most potent antioxidant suppliers and free radical scavengers [Nithiyanantham et al. 2012]. Foliar supplementations of Se and ascorbic acid (vitamin C) at appropriate concentrations (depending on the species) trigger desirable effects on plant metabolism such as inducing activities of antioxidant enzymes, increasing nonenzymatic antioxidant compounds [Ardebili et al. 2015]. Inside plants, inorganic selenium is converted to low molecular weight amino acids up to selenoproteins. These proteins are responsible for most of the physiological functions mediated by Se such as antioxidative action, redox regulation, immune function etc. [Priyadarsini et al. 2013]. Close relationship between biological properties of Se, polyphenols and other antioxidants of *A. Allium cepa* supposes the synergetic effect of different components (including Se) of antioxidant defence system display in fortified product [Golubkina 2016].

The main objective of the present work was to increase organic selenium in monitored garden pea plants by the way of foliar fortification with inorganic selenium and to observe the influence of biofortification on other qualitative parameters (total antioxidant capacity TAC and total polyphenols content TPC).

MATERIALS AND METHODS

Trial establishment. The field experiment with two varieties of garden peas (Premium and Ambassador) was established on April 22nd, 2014 (14.7°C; 0.1 mm of precipitation) and March 20th, 2015 (3.9°C; 0 mm of precipitation) in the Botanical Garden, SUA in Nitra, in three variants.

Trail variants were following:

C – control – 0 g Se ha $^{-1}$ (0 mg Se m^{-2}).

SeI – application of 50 g Se ha^{-1} (5 mg Se m^{-2}) in the form of water solution in the form of sodium selenate anhydrous (99.8+% metals basis, EEC No: 236-501-8, Alfa Aesar, Deutschland) in flowering stage.

SeII – application of 100 g Se ha^{-1} (10 mg Se m^{-2}) in the form of water solution in the form of sodium selenate anhydrous in flowering stage.

For each variety with three variants and four repetitions, there were created 24 square small fields with space of 1 m^2 per one sowing (85 plants per 1 m^2) were created. The addition per one plant was 1 ml of sodium selenate solution.

Air temperatures and precipitation were measured directly on the land of Botanical Garden of SUA and climatograms are shown in Figures 1 and 2.

Before planting the soil was prepared according to the technological demands of garden peas on the base of soil analyse. Before sowing the natural Se content in soil was established to 0.08 mg Se kg^{-1}. During vegetation of the pea the field trial was treated by hand hoeing, loosening and irrigation in the absence of moisture. The foliar application of the aqueous sodium selenate solution to the whole plant was realized once on Premium variety in the flowering phase on 3rd June, 2014 (14.3°C, 4.0 mm before application, followed by a dry period until harvest) and also on Ambassador under the the same conditions, on 17th June, 2014 (18.2°C; with 0 mm of precipitation to harvest) by hand sprayer. After the foliar application of selenium, only the root irrigation of the plants was realized. Premium harvesting took place on 19th June, 2014 (average air temperature from biofortification was 20.7°C, 0 mm precipitation) and Ambassador variety on 30rd June 2014 (average air temperature from biofortification was 18.4°C, 46.5 mm precipitation) in the phase of technological maturity. In the second year of cultivation, the foliar application of the aqueous sodium selenate solution to whole plants on the Premium variety in the phenological phase of flowering took place on 25th May, 2015 (15.1°C,

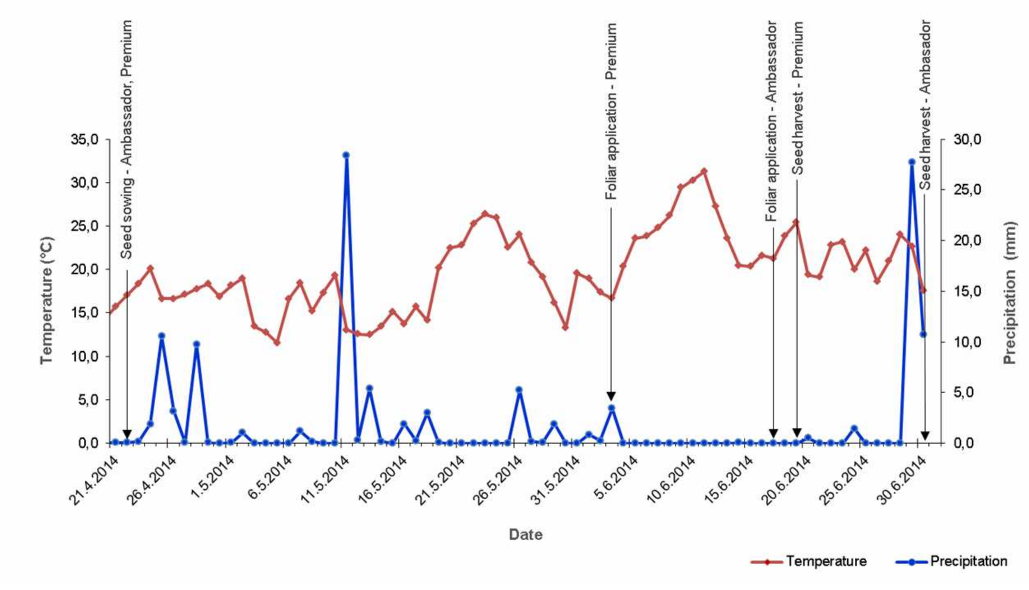

Fig. 1. Climatogam of precipitation and temperatures during growing of the garden pea in 2014

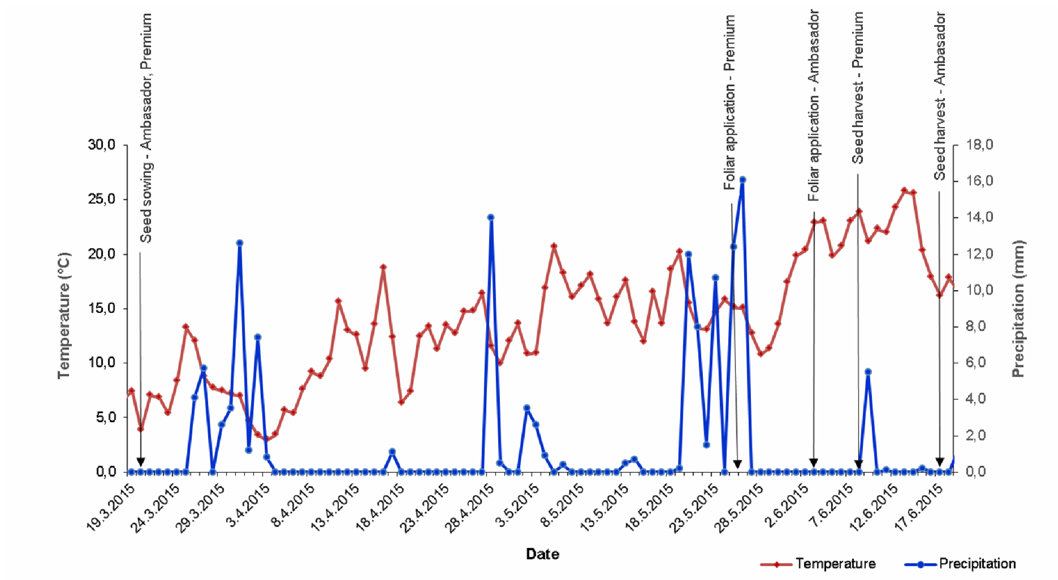

Fig. 2. Climatogam of precipitation and temperatures during growing of the garden pea in 2015

12.4 mm of precipitation, and the on the other day 16.1 mm) and on the Ambassador variety on 3rd June, 2015 (22.9°C; 0 mm of precipitation to the harvest) by hand sprayer. The sodium selenate solution on Premium variety was applied before to precipitation, and the plants were covered with a translucent foil to prevent the selenium washing from the plant by the rainwater. The harvest of Premium variety was realized on 8th June, 2015 (average air temperature from biofortification was 18°C, 28.5 mm precipitation) and of Ambassador variety on 17th June, 2015 (average air temperature from biofortification was 21.9°C, 5.8 mm precipitation) at the stage of technological maturity.

The selenium content, the total polyphenol content and total antioxidant capacity were determined in lyophilised seeds of garden pea.

Characterisation of garden pea varieties

***Pisum sativum* L. var. Premium** – is an early variety, which needs 680 thermal units for its maturing. Plant height is 60–65 cm. The first pods are on 9–10 node and the number of pods on the node is 1–2. The length of the pods is 8–9 cm; the pods are straight and blunt-ended. Number of seeds in the pod is 7–8, seed colour is dark green and calibration of grain is moderate. Weight of 1000 seeds is about 220 g. It belongs between the suitable varieties of garden peas for industrial processing, as well as for gardens. It has high resistance to *Fusarium oxysporum*.

***Pisum sativum* L. var. Ambassador** – is a medium late variety, for the maturing it needs 845 thermal units. Plant height is 75–80 cm. The first pods are formed at 15–16 node, their number is 2. The length of the pods is 8–9 cm, are straight and blunt-ended. Number of seeds in the pod is 8–9 and grain colour is dark green. Weight of 1000 seeds is about 200 g. He has medium resistance to mosaic virus pea and mildew, high resistance to the bean yellow mosaic virus, and *Fusarium oxysporum*. This variety of garden pea is suitable for industrial processing and even in gardens.

Determination of qualitative parameters

Selenium content. Mineralization of the plant material took place in the microwave mineralizer type CEM Mars X – press (microwave digestion oven). In the mineralization container there was weighed 0.5 g of the sample. It was wetted with 1 ml double distilled water followed by addition of 5 mL of conc. HNO_3 (67%) and 1 ml of H_2O_2 (30%). It was mineralized at 150°C for a period of 20 minutes. The mineralization product was refilled in to volumetric flask till 25 ml. Quantitative determination of Se was done by using of ET-AAS method with Zeeman background correction. Atomic absorption spectrometer SpectrAA240FS (Varian, Mulgrave Virginia, Australia) was used to measure the total selenium content [Hegedűs et al. 2008]. Conditions for selenium measurement were set in the equipment according to the recommendations of the manufacturer [Rothery 1988] for ET-AAS technique. In the research, chemicals with analytical purity were used.

Total polyphenol content (TPC). Total polyphenols were determined by the method of Lachman et al. [2003] and calculated on mg of gallic acid equivalent (GAE) per kg dry mater (DM). Gallic acid is usually used as a standard unit for phenolic content determination because a wide spectrum of phenolic compounds. The total polyphenol content was estimated using Folin-Ciocalteau assay. The Folin-Ciocalteau phenol reagent was added to a volumetric flask containing 100 µL of extract. The content was mixed and 5 mL of a sodium carbonate solution (20%, w/w) was added after 3 min. The volume was adjusted to 50 mL by adding of distilled water. After 2 hours, the samples were centrifuged for 10 min and the absorbance was measured at 765 nm of wave length against blank (spectrophotometer Shimadzu UV/VIS – 1240). The concentration of polyphenols was calculated from a standard curve plotted with known concentration of gallic acid.

Total antioxidant capacity (TAC) with DPPH method. Total antioxidant capacity was measured by the Brand-Williams et al. [1995] method-using a compound DPPH (2.2-diphenyl-1-pikrylhydrazyl). Compound $DPPH^{\cdot}$ was pipetted to cuvette (3.9 m^3), then the value of absorbance which corresponded to the initial concentration of $DPPH^{\cdot}$ solution in time A_0 was written. Then 0.1 cm^3 of the followed solution was added and then 2. was immediately started to measure 1. the dependence A = f(t). The solution in the cuvette 2. was mixed and measured 1. the absorbance of 1; 5 and 10 minutes at 515.6 nm in the spectrophotometer (Shimadzu UV/VIS – 1240). The percentage of inhibition reflects how antioxidant compound are able to remove $DPPH^{\cdot}$ radical at the given time.

$$\text{Inhibition (\%)} = (A_0 - A_t / A_0) \times 100$$

Total antioxidant capacity (TAC) with photochemiluminescence (PCL) method. Total antioxidant capacity was evaluated on the basis of photochemiluminescence (PCL). The PCL assay, based on the method of Popov and Lewin [1994], was used to measure the antioxidant capacity of extracts with a Photochem instrument (Analytik Jena AG, Germany) against superoxide anion radicals generated from luminol, a photosensitizer when exposed to UV light. Chemiluminescence evolution was monitored by PCLsoft control and analysis software. Lag time (seconds) was used as the radical scavenging activity. Antioxidant capacity estimated by comparison with a trolox standard (0.05–6.0 µg mL) was expressed as grams of Trolox equivalent (TE) per kilogram of sample. Antioxidant index was obtained by dividing the antioxidant capacity by lag time multiplied by 1000 (antioxidant activity/lag time × 1000) [Oomah et al. 2006, Oomah et al. 2008]. Phenolic extracts were microfuged (5 min at 14000 rpm) prior to analysis.

A statistical analysis was performed using the Statgraphic Centurion XVII (StatPoint Inc. USA). Obtained results were evaluated by analysis of variance (ANOVA), the multifactor analysis of variance (MANOVA) and the multiple Range test.

RESULTS AND DISCUSSION

Selenium content

Based on the results, the foliar application of solutions of selenium compounds appears to be more advantageous form of green plants selenization. One of the plant's specific abilities is the assimilation of inorganic selenium and its subsequent transformation into organic selenium. A solution of a selenium compound (usually selenite or selenate) is applied directly to the plant, with reducing of its required amount and, consequently, the risk of excessive (uncontrolled) selenium accumulation in the soil. The effectiveness of this method is comparable to the soil fortification with selenium compounds.

Table 1. Selenium content in observed pea varieties and variants, Nitra*

Selenium content ($\mu g\ g^{-1}$ DM)	Ambassador 2014	Ambassador 2015	Ambassador[A] Average 2014–2015
C	0.08 ±0.01a	0.12 ±0.02a	0.10 ±0.02a
Se I	2.03 ±0.31b	0.29 ±0.11a	1.16 ±0.21b
Se II	3.93 ±2.38c	0.64 ±0.41b	2.29 ±1.40c
	Premium 2014	Premium 2015	Premium[A] Average 2014–2015
C	0.07 ±0.01a	0.11 ±0.03a	0.09 ±0.02a
Se I	1.65 ±0.21b	0.96 ±0.26c	1.30 ±0.24b
Se II	3.25 ±0.27c	1.19 ±0.13d	2.22 ±0.20c

* Means ± standard deviation. Values in columns with different letters are significantly different at $P < 0.05$ by LSD in ANOVA

Our results listed in Table 1 show that the selenium content in the growing season 2014 increased statistically significantly in pea seeds of both studied varieties. In the Ambassador variety, the foliar application of 5 mg Se m^{-2} to the plant resulted in a 25.4 fold increase in its seed content (2.03 $\mu g\ g^{-1}$ DM) and a dose of 10 mg Se m^{-2} even 49.1 fold (3.93 $\mu g\ g^{-1}$ DM), what indicates its increase depending on the applied doses. Foliar application of selenium resulted in a similar increase in the content of selenium in Premium seeds, namely 23.6 fold (1.65 $\mu g\ g^{-1}$ DM) and 46.4 fold (3.25 $\mu g\ g^{-1}$ DM), depending on the applied doses. The increase in selenium content was statistically significant for both studied varieties. Comparing two varieties, it was found that in seeds of the early Premium variety had accumulated about 18.7% (after application of 5 mg Se m^{-2}) and about 17.3% (after application of 10 mg Se m^{-2}) less selenium than in the middle early Ambassador variety. Foliar application of selenium in the cultivation period 2015 increased its content in both varieties similarly as in the previous year, but significantly lesser than in 2014. In the seeds of Ambassador variety, the foliar application of 5 mg Se m^{-2} resulted in only 2.6 fold (0.29 $\mu g\ g^{-1}$ DM) increase in its content and 10 mg Se m^{-2} approximately in 5.3 fold increase (0.64 $\mu g\ g^{-1}$ DM), which is approximately 10 times lower than in 2014 (tab. 1). Foliar application of selenium in seeds of Premium variety has resulted in a more pronounced cumulating of selenium than in the Ambassador variety. A dose of 5 mg of m^{-2} increased 8.7 fold (0.96 $\mu g\ g^{-1}$ DM) selenium content in seeds, and a higher dose (10 mg Se m^{-2}) 10.8 fold (1.19 $\mu g\ g^{-1}$ DM) what in comparison with 2014 is about 3 to 4 times lesser. The increase in selenium content was also statistically significant in 2015 for both studied varieties. In Table 1 are also listed the average values of selenium content of garden pea seeds grown in 2014 and 2015. The most effective was the double dose of Se (100 g ha^{-1}), where for Ambassador there was noticed increasing from 0.10 (control) to 2.29 $\mu g\ g^{-1}$ dry matter (DM) (23 multiply increasing), and for Premium from 0.09 to 2.22 $\mu g\ g^{-1}$ DM (25 multiply increasing) when following the average values from both monitored years.

It is assumed that differences in selenium content in pea seeds between two growing seasons could have been caused by different climatic conditions. In 2015, sowing was realized a month before the date of sowing in 2014, and the application of selenate to the Premium variety was 9 days earlier, and on the Ambassador variety 14 days earlier than 2014. The air temperature during the sowing in 2015 was only 3.9°C, while in 2015 it was even 14.7°C. Air temperatures during the application of selenate on Premium variety were approximately the same during both cultivation years and during the fortification of Ambassador variety in 2015 they were about 5°C higher. Atmospheric precipitation on the day of application of selenate and on the next day after application in 2015 could not result in plant selenium washing, as it was covered by a translucent foil before rainfall. In 2015, the month June was extremely dry compared to 2014, which was warm. In the Ambassador variety, the selenium content reduction was more pronounced, when a higher temperature (23°C) during application of selenate solution could cause as the stress factor when comparing to the Premium application (15°C). On the basis of the obtained results, it is possible to formulate a hypothesis that, from the point of view of the physiological state of the plants, it is preferable to perform the sowing later, at higher air temperatures (about 14°C), and to apply the selenate solution at temperature 14–15°C. Atmospheric air temperature may be one of the stress factors. When organisms are stressed they require more energy to produce ATP and O_2, their consumption is increased in mitochondria [Bartoli et al. 2005]. The first step of selenate assimilating in chloroplasts is its reduction and activation by ATP sulphurylase to adenosine phosphoselenate (APSe), which represents the activated form of selenate. The enzyme ATP sulphurylase determines the restriction of selenate reduction, and at the same time it regulates the accumulation of selenium. Selenate is reduced to selenide to form selenocysteine (SeCys), which can then be converted into selenomethionine (SeMet), and methylated metabolites including Se-methylselenocysteine (methylSeCys), and dimethylselenide [Terry et al. 2000, Pilon-Smits and Quinn 2010]. Methylated forms of selenium are volatile and they can escape into the atmosphere by volatilization.

Authors Smrkolj et al. [2006] also conducted experiments with foliar application of Se on pea plants. The addition per plant was 0.9 ml of sodium selenate solution with a concentration of 10 mg dm^{-3}. The solution was applied to plants during the flowering period. The average Se content in the control seeds sample was 0.021 and in the leaves 0.041 μg g^{-1}. Once sprayed plants had a Se content in seed on level of 0.383 μg g^{-1}, and in case of twice sprayed plants it was double – 0.743 μg g^{-1}. In the seeds of garden pea, pumpkin and buckwheat Smrkolj et al. [2006] estimated as the major compound the selenomethionine (SeMet), which represented 82% of the total Se content in pea, 81% and 93% of the total selenium content in pumpkin seeds and buckwheat.

In our experiments after the foliar application of 10 mg Se m^{-2} (1 ml per plant) on the other pea varieties in 2015 we also achieved higher contents in pea seeds (Ambassador – 1.7 times more, Premium – 3.1 times) as authors Smrkolj et al. [2006]. Various plant species which have been treated with solutions of selenium compounds have been described in the literature, using a wide range of concentrations from 20 to 100 g Se ha^{-1} [Maksimovic et al. 1998, Qiuhui et al. 2000, Ducsay et al. 2006]. Increasing of selenium content in leaf vegetables was estimated by Simojoki et al. [2003], which reported 39 mg of Se kg^{-1} DM in lettuce leafs in a variant with the addition of 1 mg per cultivation container, what represents a 1260 fold increase compared to the control (0.031 mg Se kg^{-1}). In the roots the selenium content was increased to 42 mg Se kg^{-1} DM, what corresponds to a 545-fold increase comparing to the control (0.077 mg Se kg^{-1}). By a dose of 50–100 g Se ha^{-1} the selenium content of tea leaves can increase up to 0.32–1.45 μg g^{-1} already after 8–26 days after application [Qiuhui et al. 2000, Maksimovic et al. 1998]. Foliar application of ascending selenium doses (0.5 to 20 g Se ha^{-1}) was also carried out in a form of sodium selenite solution on winter wheat at the end of the tillering [Ducsay et al. 2006]. The applied selenium doses (0.5 and 1 g Se ha^{-1}) did not cause statistically significant increase of its content in grain which varied at the level 0.047 and 0.062 mg Se kg^{-1}. Foliar application 10, resp. 20 g Se ha^{-1} has increased statistically the selenium content in wheat gains to 0.094 resp. 0.192 mg kg^{-1} compared to variant without applied selenium.

The content of selenium in the soil, when phytotoxicity is started to demonstrate, is different for different types of crop plants. It depends on several factors, from the growth phase, the physiological state of the plants, the chemical form of accumulated selenium and other factors [Terry *et al*. 2000]. This fact was also observed by authors who confirmed in plants with low Se accumulative capacity its borderline concentration of phytotoxicity in tissue of the shoots more than 2 mg Se kg^{-1}, and in rice up to 330 mg Se kg^{-1}[Merian 1991, Terry et al. 2000]. The problem of phytotoxicity was also reported by Hegedűs et al. [2005]. Statistical evaluation in changes of total phytomass and pea seeds after addition of 2 mg Se kg^{-1} in soil was a significantly lower pea grain weight at time of harvest compared to the control variant. This phenomenon has been explained as the braking effect of applied sodium selenate on plants.

Also the results of other authors proved dependency of Se content increasing on fertiliser dose. According to Jiang et al. [2015], Se accumulation in common buckwheat was closely associated with the application rate of Se, similarly in case of Germ et al. [2013] the increase in Se concentrations in the grain of the double fortified plants was 6-fold greater in comparison to the Se-only bio-fortified plants. According to Poblaciones et al. [2013] the difference in concentration between selenite and selenate was more evident as the dose increases. The relationship between the total Se concentration in grain of the pea and the Se doses (0, 10, 20, 40, 80 g ha^{-1}) was linear and highly significant ($p < 0.001$) for both Se forms. For each gram of Se fertilization as sodium selenate or sodium selenite, the increase of total Se concentration in the grain was 148 and 19 μg Se kg^{-1} dry weight, respectively. Ingestion of 100 g of peas previously fertilized with 10 g of sodium selenate per hectare would result in an intake of 179 μg of Se. Similarly one year later, where they applied sodium selenate and sodium selenite in biofortification programme on bread making wheat plants, the results showed a strong and linear relationship between total Se in grain and Se dose for both fertilisers, although selenate was much more efficient Poblaciones et al. [2014]. In Australia biofortificated the lentil and found out that a total of 40 g ha^{-1} foliar application of Se during the reproductive stage increased seed Se concentration from 201 to 2772 μg kg^{-1} [Rahman et al. 2015]. An inorganic form of Se after metabolic process in plants becomes organic with very high bioavailability for animals and people, which was confirmed by the results of Yan and Johnson [2011]. The overall bioavailability was approximately 88% for Se from yellow peas and 92% from oats. It was concluded that Se from naturally produced high-Se yellow peas or oats is highly bioavailable in this model and that these high-Se foods may be a good dietary source of Se. According to Giacosa et al. [2014] the intake for 20 days of a daily portion (80 g) of selenium enriched rice, obtained by foliar fertilization with sodium selenate, is associated with a significant increase of serum Se levels and of GPx (Glutathione Peroxidase) activity. Followed our results showed in Table 2 it can be said that by the consumption of 100 g of dried garden pea seeds Ambassador variety and 50 g Premium variety (with lower content of selenium from the year 2015) fortified by 100 g Se ha^{-1} the daily recommen-

Table 2. Total polyphenol content in observed pea varieties and variants, Nitra*

Total polyphenol content (mg GAE kg^{-1} DM)	Ambassador 2014	Ambassador 2015	Ambassador^{A} Average 2014–2015
C	1371 ±16c	1572 ±183a	1471 ±100a
Se I	1420 ±26d	1961 ±486b	1690 ±256ab
Se II	1424 ±15d	3049 ±203d	2237 ±109c
	Premium 2014	Premium 2015	Premium^{B} Average 2014–2015
C	1378 ±25c	1456 ±77a	1417 ±51a
Se I	1278 ±15b	1591 ±272a	1434 ±143a
Se II	1203 ±25a	2559 ±257c	1881 ±141b

* Means ± standard deviation.
Values in columns with different letters are significantly different at $P < 0.05$ by LSD in ANOVA

ded dosage (55 µg d^{-1}) for humans [Elmadfa 2009] can be covered. Changes in selenium content in preserved peas were followed by Hegedűs et al. [2010]. They used heat preserving in salted brine after previous grain blanching. As the experimental material the garden pea was used, cultivated on soil treated and untreated with sodium selenate, respectively from manufacturing technology without soil selenization. After preservation the samples were opened for analysis after two months of storage. The results of the analyses indicate the partial extraction of selenium from the pea seeds to the brine, but a substantial part (77%) remains in the seeds. This means that when only the canned pea seeds itself are consumed, a substantial part of the produced selenium also gets into the organism in that case.

Total polyphenol content (TPC)

Phytochemicals, especially polyphenols, have an ideal structure for free radical uptake. Polyphenols are not considered as nutritionally valuable substances, but for their active antioxidants properties with beneficial effects on human health the interest in them is increasing. In significant sources of phenolic compounds also legumes can be considered. In garden pea the saponins, flavonoids, phenolic acids, and protease inhibitors are represented as secondary metabolites, the lecithin is also significant. According to scientific studies, green peas occur between crops with high antioxidant activity. This potential is mainly associated with polyphenols [Hegedűsová et al. 2015].

From our results listed in Table 2 results that TPC content in the growing season 2014 was increased statistically significantly in pea seeds of the Ambassador variety and decreased slightly in seeds of Premium variety. In the early variety Premium foliar application in doses of 5 mg Se m^{-2} and 10 mg Se m^{-2} per plant resulted in 7.3% and 12.7% reduction in TPC content in pea seeds (1278 mg GAE kg^{-1} DM and 1203 mg GAE kg^{-1} DM). Conversely, foliar application of selenium produced a slight, approximately equal increase in TPC content in seeds of middle early variety Ambassador, to 3.6% (1420 mg GAE kg^{-1} DM) and 3.9% (1424 mg GAE kg^{-1} DM) in dependence to applied doses. In the growing year 2015, compared to 2014, the TPC content increased significantly in case of both varieties, but also in the control variant. In the seeds of Ambassador variety, the foliar application of 5 mg Se m^{-2} resulted in 24.7% (1961 mg GAE kg^{-1} DM) increase in TPC content and 10 mg Se m^{-2} even to 94% increase (3049 mg GAE kg^{-1} DM), what is approximately 2 times more compared to 2014. A dose of 5 mg Se m^{-2} increased the TPC content of Premium seeds about 9.3% (1591 mg GAE kg^{-1} DM) and a higher applied dose (10 mg Se m^{-2}) even to 75.8% (2559 mg GAE kg^{-1} DM), which is approximately 2 times more compared to year 2014.

When evaluating the average data from both years and impact of Se application there was found significant positive influence on TPC content in case of both varieties after 100 g ha^{-1} selenium application (tab. 2). Total polyphenol content ranged in interval from 1471 up to 2236 mg GAE kg^{-1} DM for Ambassador variety and from 1417 to 1880 mg GAE kg^{-1} DM for Premium in dependence on observed variant. Mentioned values presents increasing of 15% (after application of 50 g Se ha^{-1}) and 52% (after application of 100 g Se ha^{-1}) in variety Ambassador and moderate increase in the variety Premium (1% and 33%).

The results corresponds with the Hegedűsová et al. [2015] monitored six pea varieties. The highest value was reached in case of variety Jumbo 1179.9 mg kg^{-1}, the lowest value in case of Premium 674.5 mg kg^{-1}. Other authors mentioned similar values for pea species. According to Han and Baik [2008] total phenolic content in case of green pea was estimated on 1200 mg kg^{-1} and for other legumes the phenolic content was 12 mg g^{-1} in lentils, 2.2 mg g^{-1} in chickpeas, 2.3 mg g^{-1} in soybeans, 2.5 mg g^{-1} in yellow peas and 1.2 mg g^{-1} in green peas. Fratianni et al. [2014] states for lentils variety San Gerardo 1098 µg g^{-1}, Colliano 1594 µg g^{-1} and for chickpea Sassano 147 µg g^{-1}, Castelcivita 183 µg g^{-1}.

Our results are in contrary with experiments of Kavalcová et al. [2014a], suggested that doses of the Se did not have significant effect on the content of polyphenols. In the trial with onion they applied Se in the form of sodium selenate in different form, there was applicated into soil. The results correspond with the decreasing in polyphenols after Se treatment (100 μmol L^{-1} selenite and selenate) in case of Tian et al. [2016] where they tested its influence on selected parameters in broccoli sprouts. The treatment did not influence the total GSL (glucosinolate) and ascorbic acid contents; significantly increased the myrosinase activity and sulforaphane, anthocyanin and flavonoids contents; and decreased the total phenolics content.

Statistically significant differences among cultivars in terms of phenolic content ($P < 0.05$) in blackberry were found according to Gündoğdu et al. [2016]. The cultivars showed different tendencies for antioxidative properties (antioxidative capacity, total polyphenols, phenolic compounds) of tested Hungarian and Persian walnut cultivars [Bujdosó et al. 2016]. For pea there were statistically evaluated results in Hegedűsová et al. [2015] where within the six observed varieties, differed by ripening, there was estimated significant difference only in case of garden pea varieties early – middle late. Following to Amarakoon et al. [2014] significant genotypic and environmental variation was not observed ($P > 0.05$) with respect to concentrations of phenolic in field pea. Similar, according the results of Timoracká et al. [2010] the differences of flavonoid contents in individual pea varieties were not significant. In contrary significant correlation was found between the content of total polyphenols and Se in the range of seven varieties (wheat einkorn, emmer wheat and spring wheat) analysed in the two years ($r = 0.709$) and also for the total range of all analysed varieties from the year 2009 ($r = 0.601$) according to Lachman et al. [2011].

Total antioxidant capacity by DPPH and PCL methods

The antioxidant effect of selenium consists in the formation of complexes, so called selenium proteins – selenoproteins, in which it is incorporated in the form of selenium amino acids, mainly selenocysteine and selenomethionin. Selenoproteins capture, respectively neutralize free oxygen radicals to prevent oxidative stress and short-term damage of the tissue and the body. The beneficial effect of Se on plants is usually ascribed to its ability to enhance TAC of their cells [Hasanuzzaman et al. 2012].

From our results listed in Table 3 results that the TAC, expressed as a % of inhibition, in the growing season 2014 was increased in garden pea seeds of Ambassador variety and it was degreased slightly in the seeds of Premium variety. In the early variety Premium, the foliar application of doses of 5 mg Se m^{-2} and 10 mg Se m^{-2} per plant resulted in 0.54% and 0.63% difference in TAC reduction in pea seeds compared to control. Conversely, foliar application of selenium caused a slight, approximately equal increase of the TAC content in seeds of the middle early Ambassador variety, about 0.8% and 1.1%, de-

Table 3. Total antioxidant capacity in observed pea varieties and variants, Nitra*

Total antioxidant capacity (%)	Ambassador		Ambassador^A
	2014	2015	Average 2014–2015
C	10.03 ±0.94c	8.33 ±1.07a	9.18 ±1.01b
Se I	10.91 ±0.70d	9.12 ±1.58ab	10.01 ±1.14c
Se II	10.39 ±0.48cd	9.41 ±1.28b	9.90 ±0.88c
	Premium		Premium^B
	2014	2015	Average 2014–2015
C	9.05 ±1.44b	8.63 ±1.03ab	8.84 ±1.23ab
Se I	8.51 ±1.01a	11.58 ±2.54c	10.05 ±1.77c
Se II	8.42 ±0.59a	8.56 ±2.46ab	8.49 ±1.52a

* Means ± standard deviation. Values in columns with different letters are significantly different at $P < 0.05$ by LSD in ANOVA

Table 4. Total antioxidant capacity in observed pea varieties and variants, Nitra*

Total antioxidant capacity (µg TE g^{-1} DM)	Ambassador		Ambassador^A
	2014	2015	Average 2014–2015
C	120.6 ±7.5a	340.5 ±30.6bc	230.5 ±19.1ab
Se I	148.5 ±4.9b	350.2 ±43.3bc	249.4 ±24.1ab
Se II	129.3 ±16.1a	284.5 ±29.7a	206.9 ±22.9a
	Premium		Premium^B
	2014	2015	Average 2014–2015
C	119.5 ±3.2a	336.3 ±45.7b	227.9 ±24.4a
Se I	230.3 ±21.9d	371.5 ±26.6c	300.9 ±24.3b
Se II	167.0 ±17.2c	372.6 ±20.1c	269.8 ±18.6ab

* Means ± standard deviation. Values in columns with different letters are significantly different at $P < 0.05$ by LSD in ANOVA

pending on the applied doses. By the PCL method there was also determined an increase of TAC for the Ambassador variety about 23% (5 mg Se m^{-2}) and 7% (10 mg Se m^{-2}), and for the Premium variety about 93% (5 mg Se m^{-2}) and 40% (10 mg Se m^{-2}) (tab. 4). Foliar application of selenium in the cultivation period 2015 resulted in a more pronounced increase of TAC content in Premium variety after application of 5 mg Se m^{-2} (about 2.95% by DPPH method) and degrease after the application of 10 mg Se m^{-2} compared to the control variant. Results from estimation by PCL method show approximately the same increase in TAC in case of both applied doses of selenium on Premium variety (10.5% and 10.8%). In the seeds of Ambassador variety, foliar application of 5 mg Se m^{-2} resulted in an increase in of TAC (as determined by DPPH and PCL) and 10 mg Se m^{-2} induced a 16.5% reduction by PCL alone. The results show that in 2015 the dose of 10 mg of Se-2 had inhibitory effect to TAC.

Average values of TAC of garden pea estimated by DPPH method were ranged in interval from 8.33 ±1.07% to 11.58 ±2.54% (tab. 4). Significant increasing of average value of antioxidant capacity of garden pea variety Ambassador by 0.83% and by 0.72% was observed after application of both dose of Se (50 g ha^{-1} a 100g ha^{-1}), in case of Premium only after application of higher dose of Se (by 1.21%). Our results of research are similar to study of Hegedűsová et al. [2015] evaluated the total antioxidant capacity in chosen six varieties of garden pea by DPPH method. They found out that the interval of total antioxidant activity ranged from 0.5% (Exzeleus) to 6.8% (Flavora). The values of antioxidant capacity of garden pea estimated by PCL method were range in interval 119.54 ±3.20 µg trolox equivalent (TE) g^{-1} up to 372.60 ±20.06 µg TE g^{-1} (tab. 5). Significantly increasing average value of antioxidant capacity of garden pea was found only in Premium variety after application of both concentration levels by 32% and 18%. Oomah et al. [2008] observed the antioxidant capacity except of other parameters in ten bean cultivars grown in southern Manitoba in 2006 using the same technique. Their results ranged from 1.6 to 11.2 µM TE g^{-1} of dry matter, what after conversion is 400.5 to 2803.4 µg TE g^{-1} content of the beans. Garden peas after selenization also had lower levels of antioxidant capacity, a maximum of 372.60 µg TE g^{-1}, what is comparable with the variety of beans Galley evaluated by Oomah et al. [2008].

Our results are in contrast with findings of Ardebili et al. [2015] tested foliar fortification and its impact on chosen antioxidants in basil plants. The foliar supplementation of Se and/or AsA (ascorbic acid), especially the mixed ones, led to significant improvement in antioxidative activities or free radical scavenging capacities, which are of importance for human nutrition and the medicinal industries. Comparing legumes from the TAC point of view, according to Petchiammal and Waheeta [2014] horse gram (brown and black), cowpea (brown), common bean and masur (black) showed high protein content and also exhibited good DPPH scavenging activity, ferric reducing and reducing power activity. Comparatively, pea (white and green) and chick pea (white, green, brown) showed lower values of antioxidant capacity as they tested the antioxidant potential of the fifteen legume seed proteins by using DPPH scavenging. In comparison with results of Kavalcová et al. [2014b], where the interval of statistically significant highest value of antioxidant capacity was recorded in onion (20.22–25.76) and statistically significant the lowest value of antioxidant capacity was recorded in garlic (4.05–5.07), the pea varieties evaluated in our trial reached the values (tab. 4) lower than onion, but higher than garlic. Comparative studies taking into account species or varieties influence together with the methods of estimation play important role. The metal-chelating activity of legumes showed a very different pattern compared with free-radical scavenging and lipid peroxidation inhibiting activities. Chickpeas and peas exhibited greater chelating activities than lentils, but were lower in free-radical scavenging and lipid peroxidation inhibiting activities [Han and Baik 2008]. Similarly in case of our TAC estimations, in case of DPPH method there was noticed significant increasing of average values in contrary to PCL method, where significant increasing of average value of antioxidant capacity of garden pea was found only in Premium variety. Halvorsen et al. [2002] also observed that TAC in peas was relatively low among legumes and vegetables. Among pulses, fava bean had the highest TAC value (1.9 mol g^{-1}) and, compared to vegetables, the TAC value in peas was similar to those in cabbage (0.9 mol g^{-1}), endive (1.0 mol g^{-1}) and aubergines (1.7 mol g^{-1}) while the highest TAC value in vegetables, 3.0 mol g^{-1}, was found in chilli pepper. When evaluating the influence of variety on TAC, in our trial there was confirmed significant difference between observed varieties in case of both methods (tabs 4 and 5). The significant influence of the year on TAC (DPPH and PCL methods) in garden pea is shown on the Figure 3 and Figure 4. This is in accordance with other authors, tested antioxidant activity in different varieties. Nilsson et al. [2004] was focused on thirty-five varieties of the green pea (*Pisum sativum* L.) which they were analysed for their total antioxidant capacity (TAC). Regarding the antioxidant capacity in both the water-soluble and the water-insoluble extract, there was a significant difference between the varieties but not between the harvest periods. Influence of variety on TAC was confirmed also for other species by various authors, f. e. by Gündoğdu et al. [2016] studied blackberry, or by Barátová et al. [2015] in case of basil with significant influence on TPC and TAC as well (estimated by DPPH method).

CONCLUSION

Selenization of garden pea plants in the flowering phase with foliar application of Se solution significantly increased Se content in seeds of both observed pea varieties. As the Slovak soils are poor in Se, which is followed by insufficient content of this antioxidant in crops, biofortification with Se seem to be one of the ways how to improve growing technologies of functional food. Moreover after selenization the content of total polyphenol and total antioxidant capacity in seeds of Premium and Ambassador varieties was increased as well, therefore garden pea seed could be used as natural source of antioxidants supporting human health.

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