OPEN ACCESS Potravinarstvo® Scientific Journal for Food Industry

Potravinarstvo, vol. 9, 2015, no. 1, p. 435-441
doi:10.5219/559
Received: 9 October 2015. Accepted: 16 October 2015.
Available online: 17 December 2015 at www.potravinarstvo.com

ISSN 1337-0960 (online)

SELENIUM CONTENT INCREASING IN THE SEEDS OF GARDEN PEA AFTER FOLIAR BIOFORTIFICATION

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ABSTRACT

Selenium plays an important role as an antioxidant in the prevention of cardiovascular disease. Content of selenium in the crops is constantly in the spotlight of professional public. Vegetables, as an important source of chemo protective substances, have irreplaceable position within the food of plant character. The aim of research work was to solve the Se content increasing in the seeds of garden pea (varieties Premium and Ambassador) through the foliar biofortification of the plants (50 g Se / ha and 100 g Se / ha) and to monitor its effect on production of photosynthetic pigments. In the seeds of fresh garden pea, the chlorophyll *a* and chlorophyll *b* content was determined by spectrophotometer depending on a variety and the doses of selenium. In lyophilized seeds there was measured content of selenium by ET-AAS methods. The statistically significant increase of selenium was confirmed with its increasing concentrations in case of both varieties. In the var. Ambassador there was increasing from 0.083 ±0.009 mg.kg^{-1} DM to 4.935 ±0.598 mg.kg^{-1} DM (60-fold) and in a var. Premium the values increase from 0.067±0.007 mg.kg^{-1} DM to 3.248 ±0.289 mg.kg^{-1} DM (48-fold) after application of 100 g Se / ha. After application of 50 g Se / ha in both varieties of peas there was reported 25-fold increasing in the selenium content in comparison with control. The content of photosynthetic pigments was also increased, or possibly left at level of un-fortificated variant (chl*a* – Ambassador – 50 g Se / ha; chl*b* – Premium – 100 g Se / ha) by foliar biofortification. Chlorophyll *a* content was high significantly increased according to used statistical methods in varieties Premium, from the content of 24.527 ±5.156 mg.kg^{-1} FM to 66.953 ±6.454 mg.kg^{-1} FM, likewise the content of chlorophyll *b* from the value of 19.708 ±5.977 mg.kg^{-1} FM to 37.488 ±6.146 mg.kg^{-1} FM (after 50 g / ha application). Foliar biofortification of different vegetable species can provide large-scale intake of minerals with antioxidant properties for human as well as an increase of certain biologically active substances as a result of their synergies.

Keywords: garden pea; foliar biofortification; selenium; chlorophyll *a*; chlorophyll *b*

INTRODUCTION

At present, there is increasing attention to the impact of nutrition on human health, mainly due to a significant increase of the number of cancer and cardiovascular diseases. Increasing of antioxidants intake has an important position in the prevention from this kind of diseases. The selenium belongs to such an elements and that's the reason of its intensive studying in the recent years. Potential positive effects of selenium on the human health are reflected especially in the fight against cardiovascular disease, since endothelial cells need enough of selenium to maximize selenium protective activities. Maintaining of optimum concentrations and activity of selenoproteins appears to be important in the prevention of the so-called lifestyle diseases **(Holben and Smith, 1999; Arthur, 2003; Zeng and Combs, 2008)**. Moreover, a low selenium intake is associated with health disorders, including oxidative stress, epilepsy, impaired fertility, immune deficiency, etc. **(Rayman, 2012; Whanger, 2004; Zeng and Combs, 2008)**. Selenium is part of glutathione peroxidase; it protects the arterial endothelium against damage of lipid peroxides. In the absence of selenium lipoperoxides accumulate in the heart, they damage the cell membrane and they are harmful in transport of the calcium, which is accumulated in a cell **(Hegedűs et al. 2007; Li et al. 2008; Rayman, 2012)**.

The level of selenium in the body depends on its concentration in the food. In to the food chain the selenium gets primarily from the soils and drinking water. Its content in plants is a function of the conditions of soil – plant system **(Arthur, 2003)**. Slovak soils are generally poor in selenium, which is related to its insufficient quantity in agricultural products. Content of selenium in the crops is constantly in the spotlight of professional public. The biological value of grown food raw materials depends on qualitative state of growing mediums - soils. Biogenic elements presented in the soils are taken by plants and thereby entering into the food chain. Plants are able to receive the inorganic selenium added to the soil (in the form of selenate and selenite) and to convert its part or all of it to the organic components. Agronomic biofortification through the application of fertilizers enriched with selenium is one of the possible ways of its content increasing in the soil. On the other hand, there is potential danger of soil contamination. Because of selenium content increasing in the edible parts of plant there is promoted its combination with other biofortification approaches, such as the foliar biofortification, i.e. selenium application directly to the plant **(Graham et al. 2007; White and Broadley, 2009)**. Foliar application was shown to be several times more efficient than application in fertilizers **(Aspila, 2005; Hegedűsová et al. 2015)**.

Selenium content in different parts of plants depends on the plant species. The concentration of selenium is two or three times higher in grain and roots as in the stems and leaves. The level of selenium achieves the values in the range from 0.001 to 0.034 $mg.kg^{-1}$ in vegetables. Crops containing the average amount of sulphur such as e.g. cabbage tend to higher Se accumulation than plants with low sulphur content **(Baghour et al. 2002)**. 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 selenium deficiency can be even more significant **(Bañuelos et al. 2013; Timoracká and Vollmannová, 2010)**.

The aim of the work was to solve the selenium content increasing in the seeds of garden pea plants through the foliar biofortification and to monitor its impact on the content of photosynthetic pigments.

MATERIAL AND METHODOLOGY

The small – area field experiment of two garden pea varieties (Premium and Ambassador) was established on 22nd April, 2014 on the Botanical Garden of Slovak University of Agriculture in Nitra. There were applied doses of selenium on two concentration levels (50 g Se / ha a 100 g Se / ha) and without selenium application (in case of control), every variant in four replications. Total area of field trials was 24 m^2. Agrochemical characteristic of soil substrate are figured in Table 1.

Before planting the soil was prepared according to the technological demands of garden peas. During vegetation of the pea the field trial was treated by hand hoeing, loosening and irrigation in the absence of moisture. Foliar application of an aqueous solution of sodium selenate was carried out on garden pea plants in the flowering stage by using of a hand sprayer. The harvest was done at the stage of consumer maturity for every garden pea variety in suitable time. In fresh garden pea seeds the chlorophyll content was determined for chlorophyll *a* as well as for chlorophyll *b*, in lyophilized seeds it was estimated the content of selenium.

Variants:

K – control without foliar biofortification

SeI – application of 50 g Se / ha in the form of an aqueous solution of sodium selenate in the stage of flowering

SeII – application of 100 g Se / ha in the form of an aqueous solution of sodium selenate in the stage of flowering.

Characterisation of garden pea varieties

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*.

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 grains 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.

Chlorophyll a and chlorophyll b content

Chlorophyll *a* and chlorophyll *b* were determined spectrophotometrically (Spektralquant PHARO 200) laterally in the acetone extract on the wavelengths λ_α = 649 nm a λ_β = 665 nm **(Hegedűsová et al. 2007).** Fresh garden pea seeds (150 – 200 g) were homogenized and 1 g of sample weight was wiped in a mortar with sea sand by the addition of 3 – 4 mL of acetone. After perfect homogenization, the acetone extract was filtered through the glass filter S4 device according to Morton. The extraction was repeated until the acetone stayed discoloured. Clear filtrate was decanted into a volumetric flask and it was filled up with 80% acetone to 50 mL. By

Table 1 Agrochemical characteristic of soil substrate.

Agrochemical characteristic	pH (H_2O)	pH (KCl)	Cox (%)	Hum. (%)	
	7.55	6.36	1.39	4.01	
Nutrients	N_{an} [$mg.kg^{-1}$]	K [$mg.kg^{-1}$]	Ca [$mg.kg^{-1}$]	Mg [$mg.kg^{-1}$]	P [$mg.kg^{-1}$]
	13.3	285.0	5630.0	364.0	252.5

the help of the measured absorbance values chlorophyll *a* and chlorophyll *b* were calculated.

Selenium content estimation

The total content of selenium was determined in a digestion of plant material patterns. Quantitative determination of selenium 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. Conditions for selenium measurement were set in the equipment according to the recommendations of the manufacturer **(Rothery, 1988)** for ET-AAS technique.

Statistical analyzes

The analysis of variance (ANOVA), the multifactor analysis of variance (MANOVA) and the multiple Range test were done using the Statgraphic Centurion XV (StatPoint Inc. USA).

RESULTS AND DISCUSSION

Evaluation of selenium, chlorophyll a and chlorophyll b content

The selenium content in the seeds of the garden pea var. Ambassador without foliar application of selenium was on the low level 0.083 ±0.009 mg.kg^{-1} of DM (Table 2). As the dry matter of garden pea is 20%, the selenium content in the fresh seeds in the control treatment is the 0.017 mg.kg^{-1} of FM. High statistically significant changes were found after foliar biofortification of the peas with selenium. The dose of 50 g Se / ha caused an increasing of the selenium content in the seeds 25-times (2.031 ±0.339 mg.kg^{-1} DM = **0.406 mg.kg^{-1} FM**), and 100 g Se / ha about 60-times (4.935 ±0.598 mg.kg^{-1} DM = **0.987 mg.kg^{-1} FM**) (var. Ambassador, Table 2).

The level of selenium content in the seeds of the garden pea var. Premium without the foliar selenium application was low (0.067 ±0.007 mg.kg^{-1} of DM) (Table 3). Highly statistically significant changes were demonstrated after foliar biofortification of the peas with selenium. The dose of 50 g Se / ha increased the selenium content in pea seeds of the var. Premium also 25-fold as well as in case of var. Ambassador (1.648 ±0.228 mg.kg^{-1} DM = **0.330 mg.kg^{-1} FM**) and the dose of 100 g Se / ha about 48 multiply (3.248 ±0.289 mg.kg^{-1} DM = **0.650 mg.kg^{-1} FM**) (Table 3). The results correspond with research of **(Kreft et al. 2013)**, where Tartary buckwheat (*Fagopyrum tataricum* Gaertn.) plants were tested. The Se was effectively assimilated by the plants and preserve into the seeds, where its concentration was more than double that in

Table 2 Selenium content, chlorophyll *a* and chlorophyll *b* content in garden pea seeds (*Pisum sativum*) – variety ´Ambassador´.

Ambassador	**selenium**	**Chl*a***	**Chl*b***	**Chl*a* / Chl*b***
	[mg.kg^{-1} DM]	[mg.kg^{-1} FM]	[mg.kg^{-1} FM]	
K	0.083 ±0.009^{a}	25.960 ±1.419^{a}	19.130 ±2.090^{a}	1.36
Se I	2.031 ±0.339^{b}	25.653 ±2.340^{a}	22.698 ±2.030^{a}	1.13
Se II	4.935 ±0.598^{c}	31.913 ±1.304^{b}	21.745 ±2.860^{a}	1.47

Note: DM = dry matter, FM = fresh matter, Means ± standard deviation. Column values with different lowercase letters in superscript are significantly different at P < 0.05 by Tukey HSD in ANOVA (Statgraphic).

Table 3 Selenium content, chlorophyll *a* and chlorophyll *b* content in garden pea seeds (*Pisum sativum*) – variety ´Premium´.

Premium	**selenium**	**Chl*a***	**Chl*b***	**Chl*a* / Chl*b***
	[mg.kg^{-1} DM]	[mg.kg^{-1} FM]	[mg.kg^{-1} FM]	
K	0.067 ±0.007^{a}	24.527 ±5.156^{a}	19.708 ±5.977^{a}	1.24
Se I	1.648 ±0.228^{b}	66.953 ±6.454^{c}	37.488 ±6.146^{b}	1.79
Se II	3.248 ±0.289^{c}	41.005 ±3.305^{b}	19.603 ±4.328^{a}	2.09

Note: DM = dry matter, FM = fresh matter, Means ± standard deviation. Column values with different lowercase letters in superscript are significantly different at P < 0.05 by Tukey HSD in ANOVA (Statgraphic).

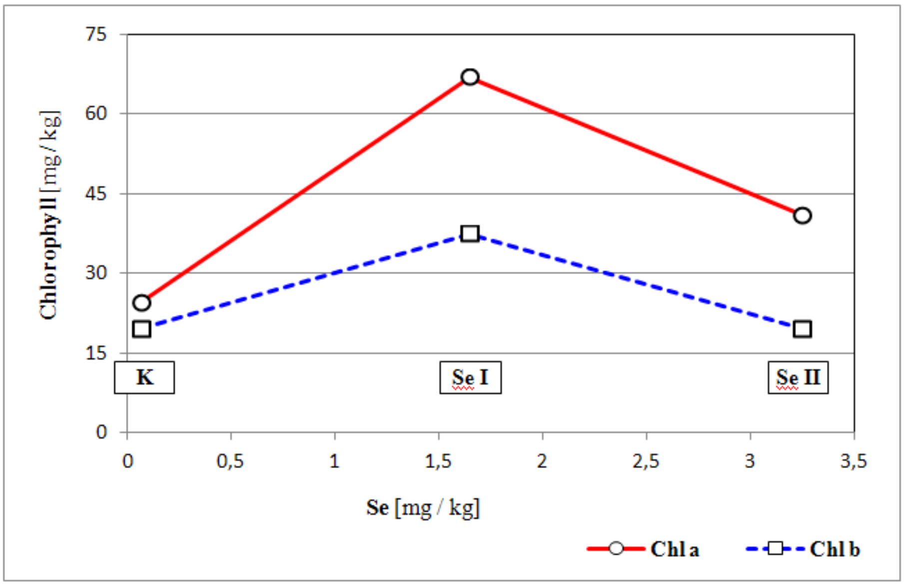

Figure 1 Dependence between of chlorophyll *a*, chlorophyll *b* and selenium content in seeds of garden pea, var. Premium.

untreated plants. Distribution of selenium and its increasing content was tested in *Fagopyrum esculentum* Moench by **(Vogrincic et al. 2009)**. The total Se content in plant parts in the untreated group was low, whereas in the Se-sprayed group it was approximately 50- to 500-fold higher, depending on the plant part. We observed a similar distribution of Se in plant parts in both control and treated groups, with the highest difference in Se content being in ripe seeds. Concerning to preserving and increasing of the selenium in to pea grains, according to **(Smrkolj et al. 2006)**, the Se content of pea seeds obtained from the untreated (UT group), once (OT) and twice (TT) foliarly treated plants, was, in each case, directly proportional to the number of spraying applications. Selenium-enriched pea seeds are a potential source of dietary selenium, on account of their ability to accumulate Se, and that this Se is present mainly as SeMet, known to be favourable for human consumption. Increasing of selenium content in peas after biofortification with inorganic form was confirmed by **(Poblacioneset al. 2013)**. For each gram of Se fertilization as sodium selenate or sodium selenite, the increase of total Se concentration in the seed was 148 and 19 μg Se / kg dry weight, respectively.

There was found the difference of selenium preserving in pea seeds within the frame of observed varieties. Seeds of var. Ambassador contain more selenium in comparison with the var. Premium, about 19% more after application of 50 g Se / ha and 34% more after application of 100 g Se / ha. This difference is probably related to a shorter growing season of var. Premium (early variety), while var.

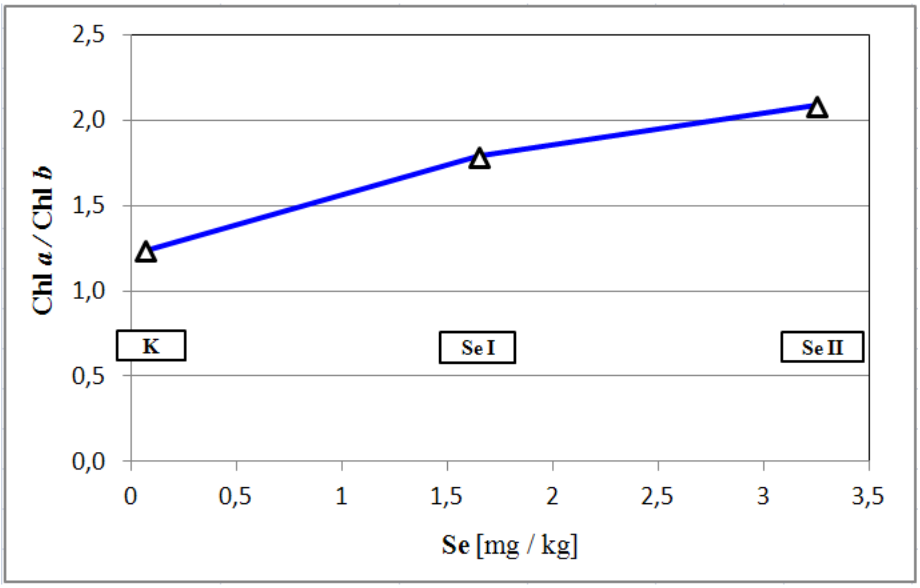

Figure 2 Dependence of chlorophyll *a* - chlorophyll *b* ratio on selenium content in seeds of garden pea, var. Premium.

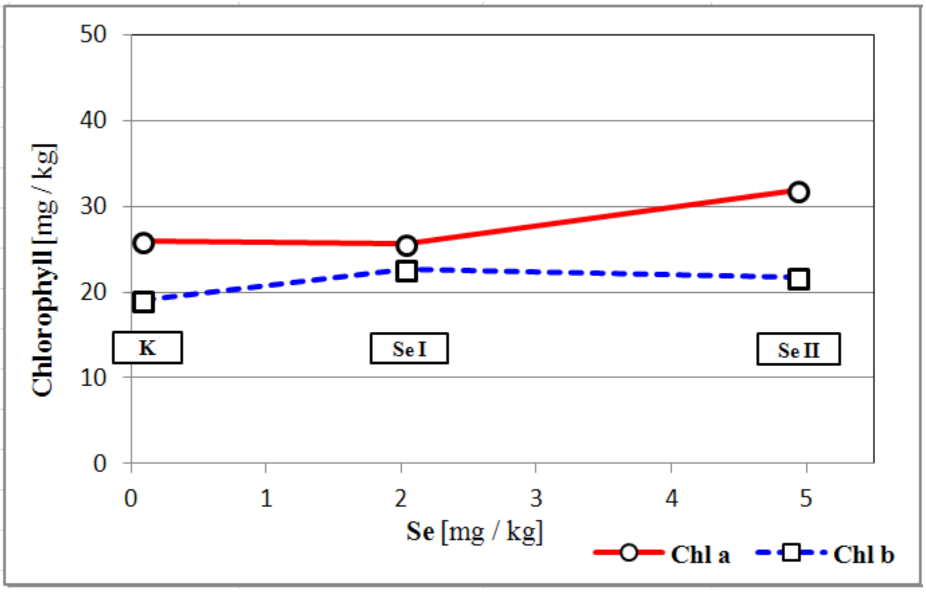

Figure 3 Dependence between contents of chlorophyll *a*, chlorophyll *b* and selenium in seeds of garden pea, var. Ambassador.

Ambassador is medium late variety.

Based on data, where average intake of selenium in Slovakia is 40 µg / person / day (0.04 mg), then after consuming of 100 g of fresh garden peas (fortified at least 50 g Se / ha) the average daily intake of selenium could be covered. Recommended daily intake of selenium for adults is 50 to 70 µg / person / day (0.05 to 0.07 mg) **(Vestník MZ SR, 1997)**. According to analysis results, after the foliar selenium application even in 50 g Se / ha, there is achieved a very significant increase of its total content in seeds, which is very important in term of biological value increasing.

Considering the mentioned deficit of daily intake, there can be said that by the addition of inorganic forms of selenium in the soil, in the form of selenate, the foodstuffs with high content of selenium can be obtained, in which selenium is bound in organic form in a significant proportion. Inside plants, inorganic selenium is converted to low molecular weight amino acids and finally into selenoproteins. These proteins are responsible for most of the physiological functions mediated by selenium such as antioxidative action, redox regulation, immune function etc. **(Priyadarsini et al. 2013)**.

Protective effects of chlorophyll are related to their ability to modulate the activation of endogenous extraneous detoxification systems and caspase of polymerase pathway, as well as with their antioxidant and antimutagenic properties **(Ferruzzi and Blakeslee, 2007)**.

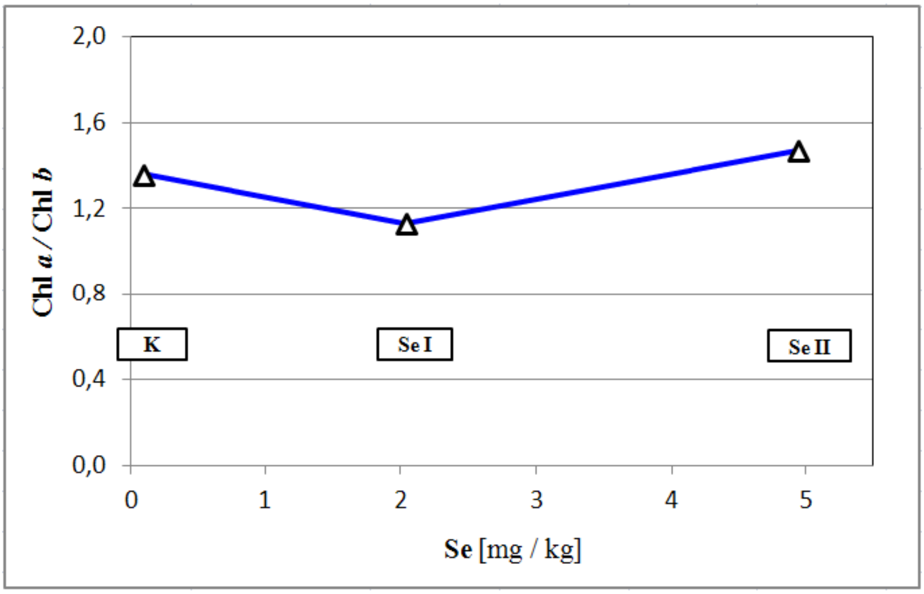

Figure 4 Dependence of chlorophyll *a* - chlorophyll *b* ratio on selenium content in seeds of garden pea.

Chlorophyll content – chl*a*, chl*b* was increasing with increased concentration of selenium, or it remained at the level of un-fortificated variants (chl*a* – Ambassador SeI, chl*b* – Premium SeII). Statistically significant changes were found after foliar application of selenium in case of var. Premium, where it occurred two - fold (100 g Se / ha) and even three - fold (50 g Se / ha) increase of chl*a* and two - fold increase of chl*b* (50 g Se / ha in comparison with control (Table 3). Increasing of the concentration of applied selenium dose from 50 g Se / ha to 100 g Se/ caused reduction of chla and chlb content (Figure 1) in case of var. Premium and increasing of the chla/chlb ratio (Figure 2). For var. Ambassador it wasn´t confirmed the statistical significance in the chl*a* and chl*b* content increasing, except of chlorophyll *a* content increasing after the application of 100 g Se / ha chl*a* in comparison with control (Table 2). Increasing of the concentration of apllied Se dose from 50 g Se/ha to 100 g Se/ha caused increasing of chl*a* content and moderate decreasing of chl*b* content (Figure 3) and increasing of the chl*a*/chl*b* ratio (Figure 4) in var. Ambassador.

Selenium significantly increased the content of chlorogenic acid, chlorophyll *a*, chlorophyll *b* and carotenoids in leaves of *Lycium chinense* **(Dong, 2013)**.

Increasing of the chl*a*/chl*b* ratio is caused by deceleration or by blocking of the chlorophyll production. Due to stress and aging the chlorophyll content is decreasing and their ratio reaches a value 3, and in the end decomposition of chlorophyll is occurring. From the ratio of chl*a*/chl*b* in our experiments for var. Ambassador there can be said that its value was almost always close to value of the control, which indicates insignificant differences in the reduction of both types of chlorophyll. In case of var. Premium the chl*a*/chl*b* ratio was the most different in case of variant with application of 100 g Se / ha comparing to control, which means that in case of this concentration it may be happened partial reduction of the chlorophylls.

CONCLUSION

Based on the evaluation of the total selenium content in the seeds of two garden pea varieties after the foliar fortification by the solution of sodium selenate there was shown statistically significant increase connected with concentration increasing of applied selenium. Results show that after consuming of 100 g seeds of fresh garden pea (fortified with at least 50 g Se / ha), the average daily intake of selenium may be covered, although there were some differences between the varieties.

The increasing concentrations of selenium resulted in an increase, in some cases statistically significant increase in the content of photosynthetic pigments (chl*a*, chl*b*), except of chlorophyll *b* content in case of var. Ambassador. From the chla/chlb ratio results that partial reduction of the chlorophylls could be occurred only in var. Premium after 100 g Se / ha application. Foliar biofortification of different vegetable species can provide large-scale intake of minerals with antioxidant properties for human as well as an increase of certain biologically active substances as a result of their synergies.

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Acknowledgement:

The work was supported by VEGA project No. 1/0105/14 and KEGA project No. 038SPU-4/2014.

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