Influence of Foliar Nutrition of Selenium on Phytochelatines Content in the Forage of Red Clover (*Trifolium pratense* L.)

Iva Klusoňová
Mendel University in Brno, Czech Republic

Pavel Horký
Mendel University in Brno, Czech Republic

Jiří Skládanka
Mendel University in Brno, Czech Republic

Markéta Komínková
Mendel University in Brno, Czech Republic

Vojtěch Adam
Mendel University in Brno, Czech Republic

See next page for additional authors

The 23rd International Grassland Congress (Sustainable use of Grassland Resources for Forage Production, Biodiversity and Environmental Protection) took place in New Delhi, India from November 20 through November 24, 2015.

Proceedings Editors: M. M. Roy, D. R. Malaviya, V. K. Yadav, Tejveer Singh, R. P. Sah, D. Vijay, and A. Radhakrishna

Published by Range Management Society of India

Presenter Information

Iva Klusoňová, Pavel Horký, Jiří Skládanka, Markéta Komínková, Vojtěch Adam, René Kizek, Petr Škarpa, and Lucia Hodulíková

Paper ID: 1105
Theme 2. Grassland production and utilization
Sub-theme 2.3. Soil-plant-animal-human interrelationships

Influence of foliar nutrition of Selenium on Phytochelatines content in the forage of red clover (*Trifolium pratense* L.)

Iva Klusoňová*, Pavel Horký, Jiří Skládanka, Markéta Komínková, Vojtěch Adam, René Kizek, Petr Škarpa, Lucia Hodulíková
Mendel University in Brno, Brno, Czech Republic
*Corresponding author e-mail: xklusono@node.mendelu.cz

Keywords: Forage, Phytochelatines, Pot experiment, Selenium

Introduction

Selenium (Se) is an essential element significantly influencing health status of animals and humans. The insufficient supply of organism with this element leads to many disorders. Conversely, higher intake can be toxic. As a part of selenoproteins, it regulates the antioxidant system and thus prevents the oxidative destruction of biological membranes and prevents the damage of the body by heavy metals. Consequently, its deficiency disrupts the overall health of animals and humans because of involvement of selenium compounds in many biological functions. Selenium concentration of plant biomass is derived from its content in the soil and may considerably vary depending on the region. One of the defense mechanisms how plants respond to the occurrence of heavy metals is the synthesis of phytochelatins. Phytochelatins (PCs) are polypeptides capable of binding the risk elements in chelate complexes. Their synthesis does not take place in ribosomes but using the phytochelatinsynthetasy enzyme. After binding of the heavy metals in chelate complexes, they are transported to the vacuoles, thereby preventing the toxic effect of metals in the cytosol. According to some authors, the synthesis of phytochelatines initiates not only the presence of heavy metals (most often cadmium) but also selenium. Red clover (*Trifolium pratense* L.) is considered as one of the most important crops. It is a perennial herb (2-4 years). As the fodder is usually grown in a pure culture or in the combination with grass or in a mixture. Forage contains a high proportion of protein and vitamins. It is fed on pasture or used to produce hay or silage.

Materials and Methods

In the experiment, red clover (Amos variety) was included. The experiment was established in the pots. Subsequently, the pots were stored in climabox. In climabox, daily temperature was set at 24 °C and 20 °C overnight, 65 % of humidity and the length of day light lasted for 12 hours (light intensity of 380 $\mu mol \cdot m^{-1} \cdot s^{-1}$). Within the first 20 days after sowing, the plants were periodically watered. For the rest of the experiment, they were automatically watered. For foliar application, the solutions of selenium at a concentration corresponding to an amount of 2; 4 and 20 $mg.m^{-2}$ of Se were used. As a source of selenium, selenite sodium or selenates were applied. Two experimental groups (selenium as selenite or selenate) and one control group (without treatment) were created. Selenium concentrations in the above mentioned forms were sprayed on 25th day on the leaf after sowing. After the application, the samples of green mass of each group were taken at a regular 14 day intervals (14th day, 28 th day and 42 nd day after the application). After the insertion into a mortar, the samples of green mass (100 mg) were disrupted by liquid nitrogen and pestle. The homogenization in bowl continued after the addition of phosphate buffer of pH 7 (0.5 ml). The prepared samples were moved to the microtube and further broken up using ultrasound for 2 minutes and vortexed for 10 minutes followed by centrifugation (25,000 g, 4 °C, 20 min). The supernatant was used for chromatographic and spectrophotometric analysis. The determination of phytochelatines was performed using high performance liquid chromatography with electrochemical detection (HPLC-ED). The results were processed in STATISTICA CZ program version 10 (Czech Republic) using a multifactor ANOVA. The differences were considered as significant with $P < 0.05$.

Results and Discussion

After the application of selenium concentration of 2 $mg.m^{-2}$, the increase ($P < 0.05$) was observed in the content of PC3 variant treated with selenate (42 nd day after the application) and selenite (14th day after the application) compared to the control variant (Fig. 1). In the content of PC4, there was no significant difference. In a concentration of 4 $mg.m^{-2}$ in the form of selenate, a significant increase ($P < 0.05$) was in the content of PC3 in all sampling terms. Conversely, the decrease ($P < 0.05$) was observed in PC3 content of selenite treated group in the 28 th and 42 nd day after the application in comparison with the control variant.

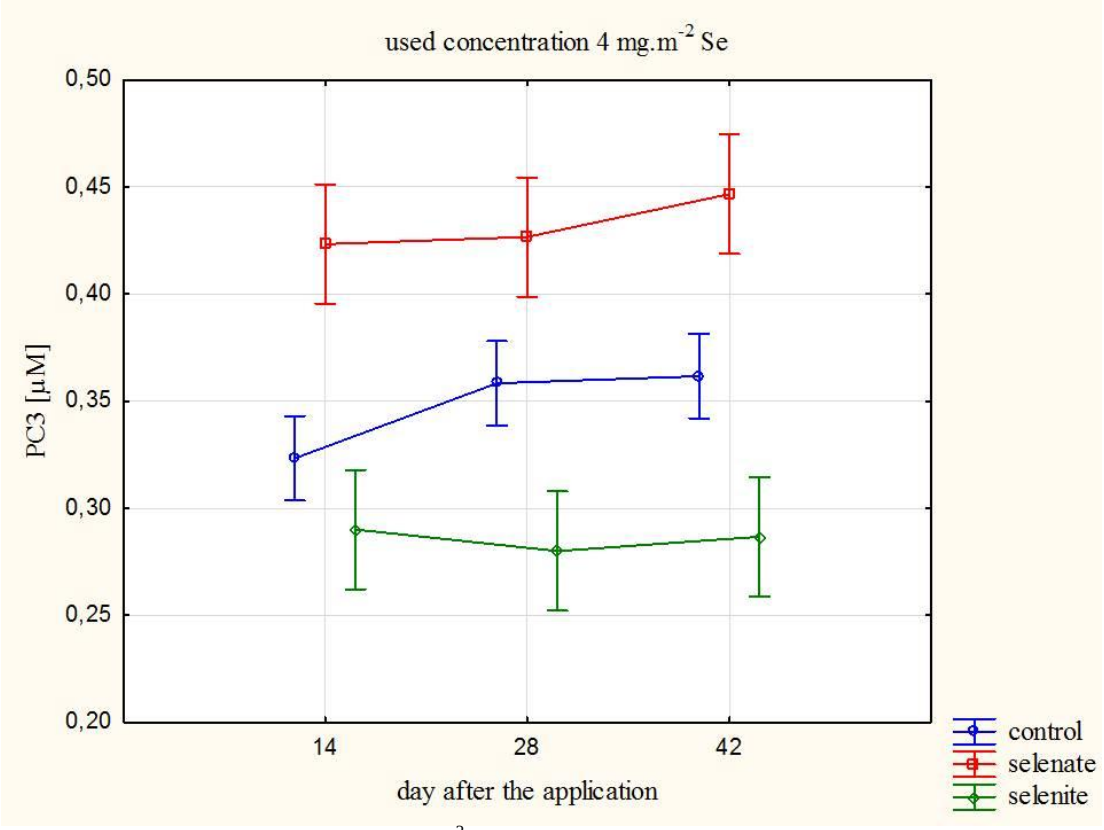

Fig. 1: Contents of PC3 after foliar application of 4 mg.m^{-2} Se

In the case of the PC4 content, a significant (P <0.05) difference between the experimental and control groups was observed. After foliar application of high selenium doses (20 mg.m^{-2}) in the form of selenate, a significant (P <0.05) increase of PC3 was found out at all sampling terms in the aboveground mass. Selenite application did not affect (P <0.05) PC3 content in plants (Fig. 2).

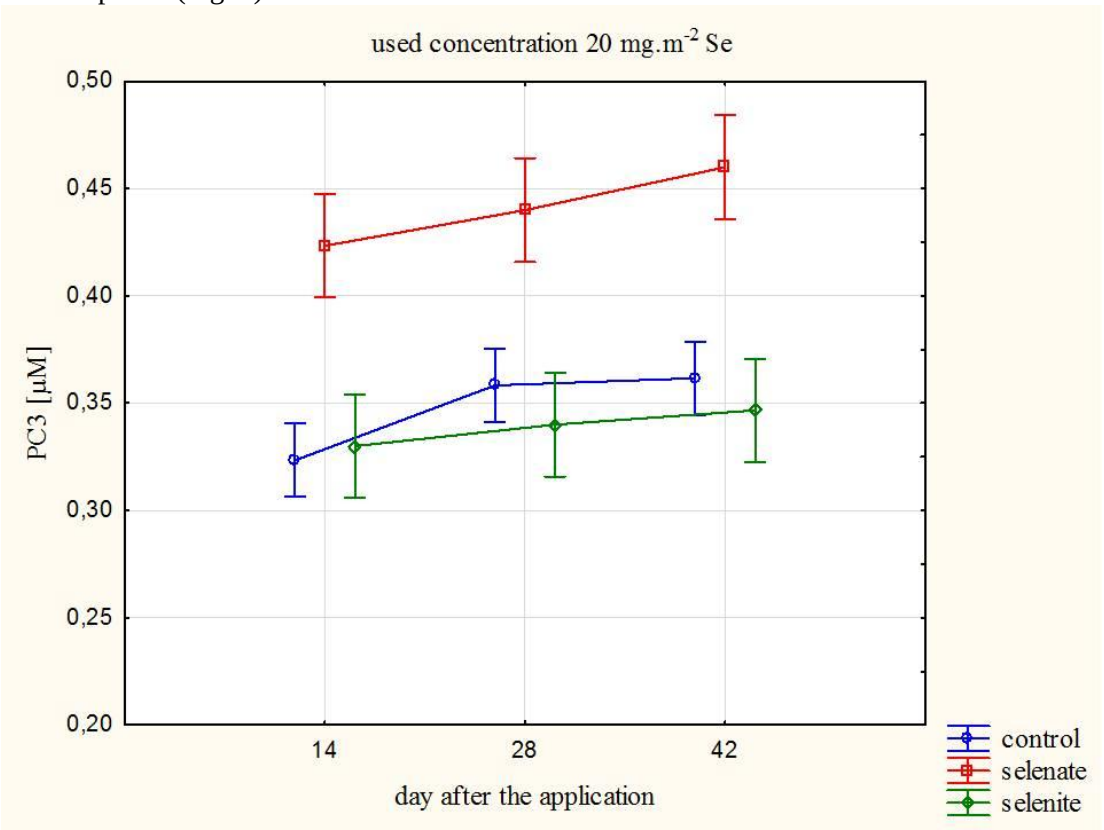

Fig. 2: Contents of PC3 after foliar application of 20 mg.m^{-2} Se

A PC4 content decreased ($P < 0.05$) after selenate treatment in the 28th day after the application in comparison with the control variant. Foliar application of selenate resulted in the increasing content of phytochelatines in aboveground mass of red clover plants. Similar results were obtained from other plant species and algae after selenium application in the soil or nutrient broth (Simmons and Emry, 2011; Krystofova *et al*., 2010). PC3 content increased in the dependence on the dose and with longer period from the application time. Hawrylak and Szymanska (2004) also recorded that phytochelatines increase the aboveground plant mass after the selenate application. Their research also shows that the plants also react to the presence of selenite. After its application, a higher content of phytochelatines can be in roots. The increased content of phytochelatines in the presence of selenite also recorded (Spain and Rabenstein, 2004). The possible reason, why there was no difference in PC4 content between control and experimental groups, may be the expression of PC4 especially in biomass of roots. On the other hand, selenium is a part of series of enzymes and proteins with an antioxidant activity and its presence in the plant can bring numerous benefits. Currently, the effect on plants is not fully understood. According to Kaur *et al*., (2014) selenium increases the resistance of plants to abiotic and biotic stress and is a growth regulator and antioxidant. Selenium supplementation of rice plants (*Oryza sativa* L.) reduces their intake of arsenic. Similar results were also achieved in plants of cucumber (*Cucumis sativus* L.) exposed to an increased concentration of cadmium. The amount of selenium deposited in animal products depends on the supply of body by feed with this element, where the organic form is more suitable than the inorganic one. Furthermore, it can be assumed that the increased level of phytochelatines rise the content of selenium in the plant, which may be desirable for feeding livestock in a selenium-deficient region. In the context with the results of other authors, selenium treating may simultaneously reduce the accumulation of heavy metals in crops intended for animal feed.

Conclusion

Foliar application of selenium as selenate resulted in the formation of phytochelatines (specifically PC3) in the aboveground mass of red clover. The value was not affected by PC4 and the plants producing phytochelatines responded to the presence of selenium in the form of selenite. The observed results indicate that foliar applied selenate in the excessive doses cause stress to treated clover plants reacting by synthesis of phytochelatines similarly to the presence of heavy metals. The clover ability to draw selenium by using the appropriate doses can be utilized to enrich crop plants grown in selenium-deficient soils and its delivery in the natural form to the feed dose of farm animals. On the other hand, this study underlines the clover's ability to cope with high doses of selenium and thus its possible utilization for phytoremediation.

References

Hawrylak, B. and M. Szymanska. 2004. Selenium as a sulphydrylic group inductor in plants. *Cellular & Molecular Biology Letters* 9(2): 329-336.

Kaur, N., S. Sharma and H. Nayyar. 2014. Selenium in Agriculture: A Nutrient or Toxin for Crops? *Archives of Agronomy and Soil Science* 60(12): 1593-1624.

Krystofova, O., V. Adam, P. Babula, J. Zehnalek, M. Beklova, L. Havela and R. Kizek. 2010. Effects of Various Doses of Selenite on Stinging Nettle (Urtica dioica L.). *International Journal of Environmental Research and Public Health* **7**(10): 3804-3815.

Simmons, D. B. D. and R. J. N. Emery. 2011. Phytochelatin induction by selenate in Chlorella vulgaris, and regulation of effect by sulfate levels. *Environmental Toxicology and Chemistry* 30(2): 469-476.

Spain, S. M. and D. L. Rabenstein. 2004. Characterization of the selenotrisulfide formed by reaction of selenite with end-capped phytochelatin-2. *Analytical and Bioanalytical Chemistry* 378(6): 1561-1567.

Acknowledgement

This project was supported by IGA MENDELU BRNO No: IP 2/2015