The impact of foliar application of mineral nutrients on the chlorophyll content in leaf and nodulation of red clover

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Abstract

Two factorial experiment with varieties of red clover K-39, K-17, Una and Viola and four foliar fertilization treatments (control, phosphorus and potassium, cobalt and boron) was set up in 2011 in Čačak. Regardless of foliar fertilization, the variety K-17 had a significantly lower chlorophyll content in leaves compared to the other cultivars. At the same time, this variety had a significantly higher number of nodules on the roots, as compared to the variety Viola. Foliar application of cobalt resulted in a significant reduction in chlorophyll content in the leaf in all of the varieties, as compared to the control. Foliar treatments cobalt and boron showed a positive effect on nodulation red clover.

Key words: red clover, fertilization, hlorophyll, nodulation.

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Introduction

In Southeast Europe, red clover (*Trifolium pratense* L.) seed crop is commonly established on acidic soils where certain macro- and micro-nutrients are less available to the plants.

Taylor and Quesenberry (1996) report that acid soils are rich in readily available forms of aluminium, iron and manganese but deficient in readily available phosphorus. Phosphorus is a constituent of all major classes of macromolecular and physiologically active compounds in the plant. It plays an important role in organogenesis, particularly in the development of generative organs (Petrović and Kastori, 1992). The low mobility of phosphorus within the plant (Koontz and Biddulph, 1957) and its high levels in the generative organs indicate the importance of the nutrient supply to plants. Potassium in plants is important for activation of enzymes, photosynthesis, synthesis and transport of different matters, transpiration, for better assimilation of the other macro- and micro-nutrients (Tiwari et al., 2001). Foliar or soil application of cobalt has a favourable effect on symbiotic nitrogen fixation in red clover (Vrany, 1978), plant growth, dry matter yield and seed yield of soybean (Reddy and Raj, 1975). As reported by Wilczek and Ćwintal (2008), a sufficient boron supply enhances pollen germination, fertilization and, hence, fertility i.e. seed number per inflorescence in red clover. Also boron has an important role in metabolism assimilation of nitrogen (Ruiz et al., 2006), but higher doses of boron have toxic effect on the plant (Ceyhan et al., 2007).

The aim of the study was to analyze the influence of foliar fertilization with phosphorus and potassium, cobalt and boron, on the chlorophyll content in red clover leaves and nodulation growing on acid soil.

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Materials and methods

The field experiment with varieties of red clover: K-39, K-17, Una and Viola and four treatments of foliar fertilizing - control, phosphorus and potassium, cobalt and boron, was established in 2011 in Cacak (43°54′39.06″N, 20°19′10.21″E, 243 m a.s.l.). The experiment was based on the alluvium soil type with acid reaction (pH_{H2O} 4,8) containing 3.18% of organic matter, 0% $CaCO_3$, 22.08 mg P_2O_5, 30.0 mg K_2O/100 g^{-1} of soil, using a randomized block design with four replications with plot size of 5 m^2 (5x1m). The primary tillage plowing was done at a depth of 30 cm and seedbed preparation to a depth of 10 cm. For basic fertilization was used complex fertilizer $N_{15}P_{15}K_{15}$ in the amount of 300 kg ha^{-1}, which is entered in the land immediately before the additional tillage in the sowing year and again in the fall after the end of the growing season. Sowing was performed on 20 cm row spacing and seed rate of 18 kg ha^{-1}. Mechanical weed control treatments were performed twice. No irrigation was used.

The mean annual air temperature in 2011 and 2012 was 12.37 °C, and 13.12 °C respectively, and the sum of annual rainfall 374.5 mm and 463.5 mm respectively. The average annual air temperature for multi-year period (1992-2002) was 11.97 °C, and the average sum of annual rainfall 680.3 mm.

In sowing year, the crop is used for forage production, and treatments and analyzes were carried out on the second regrowth in the second year of cultivation, when the seed production is the most often carried out. Foliar applications of phosphorus and potassium (P 52 K 34, Haifa, Israel in a concentration of 1%, with water usage of 1000 l ha^{-1}) was performed once, at the stage of intensive growth in the second growth in the second year of cultivation. The foliar application of cobalt ($Co(NO_3)_2$) in concentration of 0.033 g l^{-1}, with water consumption of 1000 l/ha^{-1} was applied once at the stage of intensive growth in the first cut in the second year of cultivation and twice at the stage of intensive growth in the second growth in the second year of cultivation. Boron (Boron-feed, Haifa, Israel at a concentration of 0.1% and water consumption by 1000 l ha^{-1}) was applied in the second growth during the second year of growing, in two occasions in the phase of intensive growth of plants. Analyses were carried out in the flowering stage. Nodulation is analyzed to each cultivar, where five plants were randomly selected to determine the total number of nodules up to a depth of 20 cm. Determination of chlorophyll content was performed by spectrophotometric method according to Cvijovic and Acamovic (2000). Obtained results were processed with Kruskal-Wallis's test.

Results and disscusion

Regardless of foliar fertilization, significantly lower content of chlorophyll *a* and total chlorophyll (*a*+*b*) in the leaf in comparison to other varieties had the cultivar K-17. Also, this variety according the Kruskal-Wallis test had significantly lower chlorophyll *b* content in leaves than variety Una. Regardless of foliar treatment, a significantly higher number of nodules on the roots of red clover was recorded in cultivar K-17, compared to the cultivar Viola ($P < 0.05$). Genotypic differences in the mineral nutrition in alfalfa also identified James et al. (1995). The authors explain this better or worse transferring nutrients inside the plants in some genotypes.

Foliar applications of cobalt has affected a significant reduction in chlorophyll *a* content and of the total chlorophyll in the leaf in all varieties compared to the control, as well as significantly lower chlorophyll *b* content as compared to a variant with foliar application of phosphorus and potassium. Foliar treatments with boron as well as phosphorus and potassium are not significantly affected by the change in chlorophyll content in leaves as compared with the control. A good supplement of the plants with cobalt is important for the normal conduct of a series of physiological reactions in the process of photosynthesis and cell growth (Lipskaya, 1972). At lower concentrations cobalt affects positively on the Hill's reaction with simultaneous reduction of the chlorophyll content and increase of the number of chloroplasts per unit of leaf area. According to Chatterjee and Chatterjee (2005), the excess of cobalt in tomato can lead to lack of iron. Authors claim that the adverse effects of cobalt, when in excess, can be seen in inhibition of PS2 activity and the decrease of export of photoasimilatives in the dark phase of the photosynthesis. At C4 plants, cobalt interferes with the fixation of carbon dioxide by the inhibition of the activity of enzymes involved in this process. The excess of cobalt makes it difficult for the synthesis of RNA and DNA, probably by modifying the large number of endo and exo-nucleases.

Table 1. The content of chlorophyll *a*, chlorophyll *b*, total chlorophyll content *a* + *b* in the leaf of varieties of red clover in the flowering stage and number of nodules on the roots depending on foliar treatment (Ø – control; Co – cobalt; B – boron; PK - phosphorus and potassium).

		Chlorophyll *a* (mg kg^{-1})	Chlorophyll *b* (mg kg^{-1})	Chlorophyll *a+b* (mg kg^{-1})	No.of nodules per plant
Varieties	K-39	2342.4 a	175.2 ab	2517.6 a	48.4 ab
	K-17	1586.9 b	221.9 b	1808.8 b	58.4 a
	Una	1789.2 a	247.3 a	3036.5 a	46.8 ab
	Viola	2503.3 a	247.9 ab	2751.2 a	37.8 b
Foliar treatment	Ø	2319.8 a	211.4 ab	2531.1 a	30.4 b
	Co	1559.0 b	188.3 b	1747.4 b	54.1 a
	B	2861.3 a	228.7 ab	3090.0 a	64.0 a
	PK	2481.8 a	263.8 a	2745.6 a	42.5 ab
Varieties	K-39 x K-17	*	ns	*	ns
	K-39 x Una	Ns	ns	ns	ns
	K-39 x Viola	Ns	ns	ns	ns
	K-17 x Una	**	*	**	ns
	K-17 x Viola	***	ns	***	*
	Una x Viola	Ns	ns	ns	ns
Foliar treatment	Ø x Co	*	ns	*	**
	Ø x B	Ns	ns	ns	***
	Ø x PK	Ns	ns	ns	ns
	Co x B	***	ns	**	ns
	Co x PK	**	*	**	ns
	B x PK	Ns	ns	ns	*

The values marked with different small letters in columns differ significantly on the level of $P \leq 0.05$ by Kruskal-Wallis - test; * - Kruskal-Wallis - test significant at the level of $P \leq 0.05$; **- Kruskal-Wallis - test significant at $P \leq 0.01$; *** - Kruskal-Wallis - test significant at $P \leq 0.001$; ns- Kruskal-Wallis - test is not significant.

Foliar treatments with cobalt and boron showed a positive effect on the nodulation of red clover. The foliar treatment of phosphorus and potassium had no significant effect on the number of nodules on the roots of red clover. On a positive impact of cobalt fertilization on nodulation of legumes indicate Achakzai (2007). Mengel and Kirkby (2001) indicate that the concentrations of cobalt in soil less than 0.1 mg kg^{-1} adversely affect rhizobial symbiosis. Bakken et al. (2004) found that the yield of red clover increased by 10% with the addition of cobalt in cobalt-deficient soils and the cobalt content in plants decreased with each successive phenophase of growth. Application of cobalt in the amount of 8 ppm resulted in higher nodulation and the number of effective nodules in pea (Nadia, 2006). Cobalt in an amount of 0.16 mg g^{-1} resulted in the significant increase in the number and weight of nodules, the concentration of nitrogen in nodules, content of leghemoglobine, the total production of biomass, and seed yield compared to untreated peanut plant (*Arachis hypogaea* L.). Plant provision of cobalt influenced the increased fixation of nitrogen in all kinds of *Rhizobium's*, and thus the better growth of legumes (Collins and Kinsella, 2011). Pattanayak et al. (2000) also reported that the use of cobalt in cowpea rise to a significant increase in the number of nodules per plant, number of effective nodules per plant, mass of effective nodules per plant, dry matter accumulation in plants, the number of pods per plant and seed yield per hectare. Cobalt is a component of vitamin B12 and coenzyme cobalamine and thus helps in the process of fixation of molecular nitrogen in the root nodules in leguminous plants (Palit et al., 1994).

The role of boron in nitrogen fixation in legumes is reflected in its positive influence on reproduction of the bacteria of the genus *Rhizobium* (Loomis and Durst, 1992). Bolanos et al. (1994) reported that a sufficient supply with boron leads to enhanced symbiotic nitrogen fixation in pea (*Pisum sativum* L.). According to Yamagishi and Yamamoto (1994), insufficient plant provision with boron influences the nodulation in soybean (*Glycine hispida* max.) and faba bean (*Vicia faba* L.).

Conclusions

Regardless of foliar fertilization, significantly lower clorophyll content in the leaf in comparison to the other varieties, had the cultivar K-17. At the same time, a significantly higher number of nodules on the roots of red clover was recorded in cultivar K-17, compared to the cultivar Viola ($P<0.05$). Foliar applications of cobalt has affected a significant reduction in chlorophyll *a* content and total chlorophyll in the leaf in all varieties compared to the control. Foliar treatments with boron as well as phosphorus and potassium, are not significantly influenced by the change of chlorophyll content in leaves compared with the control. Foliar treatments with cobalt and boron showed a positive effect on the nodulation of red clover. Given the positive results from this experiment, the research should continue, or to determine the optimal concentration of mineral nutrients and justification of their application in wider production.

References

Achakzai A. K. K. (2007). Effect of various levels of nitrogen fertilizer on nodulation of pea cultivars. Pak. J. Bot. 39: 1673-1780.

Bakken A. K., Synnes M. O., Hansen S. (2004). Nitrogen fixation by red clover as related to the supply of cobalt and molybdenum from some Norwegian soils. Acta. Agric. Scand., Sect. B, Soil and Plant Sci. 54: 97-101.

Bolanos L., Esteban C., De Lorenzo C., Fernandez-Pascual M., De Felipe R.M., Garate A., Bonilla I. (1994). Essentiality of boron for symbiotic dinitrogen fixation in pea (Pisum sativum) rhizobium nodules. Pl. Phisiol. 104: 85-90.

Ceyhan E., Onder M., Harmankaya M., Gezgin S. (2007). Response of chickpea cultivars to application of boron in boron-deficient calcareous soils. Communication in Soil Science, Plant and Analysis 38: 1-19.

Chatterjee J., Chatterjee C. (2005). Deterioration of Fruit Quality of Tomato by Excess Cobalt and Its Amelioration. Communications in Soil Science and Plant Analysis 36: 1931–1945.

Collins N. R., Kinsela S. A. (2011). Pedogenic factors and measurements of the plant uptake of cobalt. Plant Soil 339: 499-512.

Cvijović M., Aćamović G. (2000). Spektrofotometrijsko određivanje hlorofila. Praktikum iz Biohemije, pp. 82.

James D. W., Tindall T. A., Hurst C. J., Hussein A. N. (1995). Alfalfa cultivar responses to phosphorus and potassium deficiency: Biomass. J. Plant Nutr. 18: 2431–2445.

Koontz H., Biddulph O. (1957). Factors affecting absorption and translocation of foliar applied phosphorus. Plant Physiology 32: 463-470.

Lipskaya G. A. (1972). Accumulation of chlorophyll in chloroplasts of cucumber leaves under the effect of cobalt and manganese applied separately and together. Biol. Nauki. 15: 90-94.

Loomis W. D., Durst W. R. (1992). Chemistry and biology of boron. Bio Factors 4: 229-239.

Mengel K., Kirkby A. E. (2001). Principles of plant nutrition, 5th ed. Kluwer Academic Publishers, Dordrecht. pp. 849.

Nadia G. (2006). Increasing the efficiency of nitrogen fertilization through cobalt application to Pea plant. Research Journal of Agriculture and Biological Sciences 2(6): 433-442.

Palit S., Sharma A., Talukder G. (1994). Effects of cobalt on plants. The botanical review 60 (2): 149-173.

Pattanayak S. K., Dash D., Jena K. M., Nayak R. K. (2000). Seed treatment of green gram with molybdenum and cobalt: Effecto on nodulation, biomass production and N uptake in an arid soil. J. Ind. Soc. Soil Sci. 48: 769-773.

Petrović M., Kastori R. (1992). Ishrana biljaka. IP Nauka, Beograd, 83-86.

Reddy D. T., Ray A. S. (1975). Cobalt nutrition of groundnut in relation to growth and yield. Plant and Soil 42: 145-152.

Ruiz J. M., Rivero R. M., Romero L. (2006). Boron Increases Synthesis of Glutathione in Sunflower Plants Subjected to Aluminum Stress. Plant Soil 279: 25–30.

Taylor N. L., Quesenberry K.H. (1996). Red clover Science. Curent Plant Sciences and Biology in Agriculture 28: 44-57.

Tiwari P. S., Joshi P. O., Vyas K. A., Billore D. S. (2001). Potassium Nutrition in Yield and Quality Improvement of Soybean. Paper presented in the International Symposium on "Importance of Potassium in Nutrient Management for Sustainable Crop Production in India" held during 3-5. December 2001 at New Delhi. http://www.ipipotash.org/udocs/Potassium%20Nutrition%20in%20Yield%20and%20Quality.pdf.

Vranny J. (1978). Microbial Changes in Clover Rhizosphere after Foliar and Soil Application of Cobalt. Folia Microbiologica 23: 236-242.

Wilczek M., Ćwintal M. (2008). Effect of the methods of additional feeding with microelements (B,Mo) on the yield structure and seed yield of red clover. Electronic Journal of Polish Agricultural Universities, http://www.ejpau.media.pl/volume11/issue4/abs-05.html.

Yamagishi M., Yamamoto Y. (1994). Effects of boron on nodule development and symbiotic nitrogen fixation in soybean plants. Soil Sci. Plant Nutr. 40: 265–74.

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