Original Scientific paper
10.7251/AGRENG2201032T
UDC 633.32:665.9

FOLIAR APPLICATION OF ZINC IN THE PRODUCTION OF RED CLOVER SEED ON ACID SOIL

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ABSTRACT

Zinc is an important microelement that enters into the composition of hundreds of enzymes and thousands of proteins in the plant and animal world. The aim of the study was to analyze the effect of foliar application of zinc in acid soil on the most important seed yield components and seed yield of selected red clover (*Trifolium pratense* L.) genotypes from Serbia and Bulgaria. The experiment was established in 2012 in Čačak (Serbia) on loessivized vertisol soil type with acid reaction (pH_{H2O} 4.8). Sowing was done at a distance of 70x40 cm. There were used four different genotypes of red clover, three of which were isolated from the local population and variety of K-39. For the analysis was used the second cut in the second year of cultivation. Two treatments were used: control - no fertilization and foliar treatment with zinc in the form of $ZnSO_4$ x $7H_2O$, at a concentration of 0.2%, using 1000 L ha^{-1} of water, at the stage of intensive plant growth. Foliar application of zinc had a positive effect on the number of stems per plant, the number of inflorescences per stem, the number of inflorescences per plant and the number of flowers per inflorescence. The negative effect of foliar application of zinc has been reported on the thousand seeds weight and the fertility of flowers at the genotypes originating from Bulgaria. A significant increase in seed yield per plant, under the influence of foliar application of zinc, was observed only in the variety K-39 (for 11.4 g $plant^{-1}$), which otherwise had the highest seed yield.

Key words: *foliar fertilization, red clover, seed yield, zinc.*

INTRODUCTION

Red clover (*Trifolium pretense* L.) is one of the most important legumes for the production of high quality forage. It grows in wild forms in meadows throughout Europe and Asia. Red clover seed yield is mainly determined by the genetic basis of the variety, the ecological conditions of the area, the time of mowing, the presence of pollinator insects, as well as the interaction of the genotype and environment (Steiner et al., 1995). The high variability and genetic plasticity of the species are the result of the extremely xenogamous character of fertilisation and entomophilous pollination (Taylor and Smith, 1979). Due to the high variability and adaptability to the various environmental conditions, natural selection has produced a large number of local ecotypes, superior to the given growing conditions (Helgadottir, 1996).

Zinc (Zn) is one of the elements that has an important metabolic role in plant growth and development and therefore it is called a necessary trace element or microelement. It has a very important physiological role in all living systems such as maintaining the structural and functional integrity of biological membranes, energy production, facilitating protein synthesis and gene expression (Mosuavi et al., 2013). Zinc is part of hundreds of enzymes and thousands of proteins in plants and animals. Therefore, the quantitative and qualitative yield of crops depends on the supply of plants with zinc. Lack of zinc in nutrition of domestic animals and humans leads to the severe health complications, including impairments of growth and the immune system combined with increased risk of infections, DNA damage and cancer development (Poblaciones et al., 2017).

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. Zinc is absorbed from the soil in the form of Zn^{2+}. According to Mosuavi et al. (2013) the solubility of zinc in acidic soils is generally high, and therefore there may be a loss by leaching, leading to its deficiency in plants. However, according to Kadović and Knežević (2002), only a small part of the total Zn in the soil is accessible to plants, only a few mg kg^{-1} of soil, and often less than one mg kg^{-1} of soil. Zn adsorption increases with increasing pH and the presence of $CaCO_3$ in the soil. McBride and Blasiak (1979) indicate that the solubility of zinc in acid soils can be reduced by the presence of competing cations, primarily Al, Cu, and Fe. The solubility of zinc in acidic soil also depends on other factors such as the content of different organic fractions, calcium carbonate, phosphorus, clay minerals and temperature (Mosuavi et al., 2013). Therefore, one reaction mechanism probably cannot explain the solubility of Zn on acidic soil, but it is conditioned by numerous factors in each individual case.

The effect of zinc fertilization on growth and yield of many plants such as alfalfa, wheat, maize, barley, cotton and potato were investigated in numerous researches and increasing in yield with zinc application was observed (Kinaci and Kinaci, 2005; Mosuavi et al., 2007; Galavi et al., 2011; Xi-Wen et al., 2011; Efe and Yarpuz, 2011; Mosuavi et al., 2013).

The aim of this study was to analyze the impact of foliar application of zinc on acidic soil on the most important yield components and seed yield of selected genotypes of red clover from Serbia and Bulgaria.

MATERIALS AND METHODS

A field trial was conducted in Čačak in Serbia (43°54’39.06" N, 20°19’10.21" E, 243 m a.s.l.) in the period 2012-2013. The experiment was established on a leached vertisol (pH 4.8), which contained 3.18% organic matter, 0% $CaCO_3$, 22.08 mg extractable P 100 g^{-1} soil, 30.0 mg K 100 g^{-1} soil and 82 mg Zn kg^{-1} of soil. Prior to seeding and in autumn, 45 kg ha^{-1} N, 45 kg ha^{-1} P_2O_5, and 45 kg ha^{-1} K_2O were incorporated into the soil.

Two factorial experiment was set up according to a completely randomised block design in five replications (with 20 plants per plot at a plant spacing of 70x40cm). A total of four red clover genotypes, including the cultivar K-39 was used. Besides the K-39 cultivar (G_1), two genotypes were extracted from local populations from Bulgaria, Petrič (G_2) and Rozova dolina (G_3), as well as one genotype (G_4) isolated from the local populations in Serbia, vicinity of Aleksandrovac (Central Serbia). Two treatments were applied: control - without fertilization and foliar treatment with zinc in the form of $ZnSO_4$ x $7H_2O$, in a concentration of 0.2%, using 1000 L ha^{-1} water in the phase of intensive plant growth (BBCH 34-36). The experiment was set up according to a randomized block system with five replicates. Weeds were controlled mechanically. No irrigation was employed.

The mean annual air temperature in 2012 and 2013 was 13.12°C and 12.99°C respectively and amount of annual rainfall 463.5 mm and 582.7 mm respectively. The average annual air temperature for the multi-year period (1992-2002) was 11.97°C, and the average amount of annual rainfall 680.3 mm.

The second growth in the second year (2013) was evaluated under field conditions for the following: stem number per plant, inflorescence number per stem, and inflorescence number per plant, using a sample of five plants per plot. Laboratory evaluation included determination of: flower number per inflorescence and seed number per inflorescence (using ten randomly selected inflorescences). Fertility (ratio between seed number and total flower number per inflorescence) and thousand seed weight (based on the seeds extracted from the same sample) were calculated. Seed yield components (inflorescence number per plant, seed number per inflorescence, thousand seed weight) were used to determine seed yield per plant which was calculated as g $plant^{-1}$.

The obtained results were subjected to a two-factor analysis of variance using SPSS software (1995). The significance of differences between mean values was tested by LSD test.

RESULTS AND DISCUSSION

Foliar application of zinc affected a significant increase in the number of stems per plant only in G3 (significance of the variety / foliar application interaction) (Table 1). The genotypes did not differ significantly in the control variant in terms of the number of stems per plant, while in the zinc variant a significantly higher number of stems per plant was recorded in G3 compared to G2 and G4. The positive effect of foliar treatment may be related to the fact that the metabolism of plant hormones such as auxins decreases in the state of zinc deficiency. Zinc is an element necessary for the synthesis of tryptophan, and this is a prerequisite for the formation of auxin, and the amount of auxin is reduced in the absence of zinc (Pedler et al., 2000). According to Derakhshani et al. (2011) foliar application of zinc in the amount of 1 and 3 g of L^{-1} affected the growth of stems in *Chrysanthemum balsamita* L., while there were no significant differences between the stated concentrations. In this experiment, foliar treatment with zinc affected the increase in the number of inflorescences per stem only in G1. In the control variant, the varieties did not differ significantly in terms of the number of inflorescences per stem. In the zinc variant, a significantly higher number of inflorescences per stem was observed in G1 and G2 compared to G4. Thanks to the cumulative effect on the number of stems per plant and the number of inflorescences per stem, foliar application of zinc also affected the increase in the number of inflorescences per plant in all genotypes except G4. Varieties did not differ significantly in the number of stem per plant in the control variant.

The average number of flowers per inflorescence was 93.9, and it ranged from 77.1-124.1.

Foliar fertilization with zinc affected a significant increase in the number of flowers per inflorescence only in G3. In the control variant, a significantly higher number of flowers per inflorescence was recorded in G2 compared to G3, while in the variant with zinc G3 had the highest number of flowers per inflorescence. According to Ali et al. (2011), zinc fertilization in the amount of 0.75 kg ha^{-1} increased significantly the number of flowers in cotton, which later affected a significant increase in yield. Foliar application of zinc in this experiment did not have a positive effect on flower fertility, and in G3 it even had a negative effect. In the control variant, the cultivars did not differ significantly in terms of fertility, while in the treatment with zinc G1 had significantly higher flower fertility compared to G2 and G3, as a result of of the negative effect of zinc in G2 and G3. The content of amino acids in plant tissues and protein synthesis decreases due to zinc deficiency. One of the places of protein synthesis is the pollen tube, in the top of which the amount of zinc is up to 150 micrograms per gram of dry matter. Thus, zinc contributes to pollination by influencing pollen tube formation (Outten and OʾHalloran, 2001; Pandei et al., 2006). However, when the amount of zinc is higher than optimal, toxicity can occur in plants (Mosuavi et al., 2013). Foliar treatment with zinc, generally observed, had no effect on the number of seeds per inflorescence. However, the results of the foliar treatment / genotype interaction indicate that in the control treatment the cultivars did not differ significantly in this trait, while in the zinc treatment a significantly higher number of seeds per inflorescence was recorded in G1 compared to G2. This indicates that foliar treatment had a minimal impact on this trait in some varieties. Jevtić et al. (2007) and Wilczek and Ćwintal (2008) also show that the presence of insect pollinators has a major impact on the number of seeds per inflorescence. Also, large amounts of rainfall during flowering can significantly reduce fertility and harvest yield in relation to the potential seed yield. The negative effect of zinc was also observed on thousand seed weight in G2 and G3. The mentioned genotypes had a significantly higher thousand seed weight on the control variant compared to G1 and G4. In the variant with zinc, the genotypes did not differ significantly in terms of the weight of a thousand seeds.

Table 1. Seed yield and seed yield components of the red clover genotypes depending on foliar application of zinc (Ø without zinc, foliar treatment with zinc)

Genotype	Treatment	SP	IS	IP	FI	SI	F	TSW	SYP
G 1	Ø	34,0ab	5,0bc	172b	77,1bc	30,8ab	40,4ab	1,385b	8,46b
	Zn	42,8abc	7,44a	308a	89,4b	39,8a	44,9a	1,525b	19,84a
G 2	Ø	33,4bc	5,74abc	191b	86,3b	30,9ab	36,6ab	1,907a	11,9b
	Zn	32,0bc	6,34ab	197a	84,1bc	25,1b	29,8bc	1,318b	6,41b
G 3	Ø	30,2bc	5,36abc	182b	59,3c	24,1b	41,7ab	1,878a	7,6b
	Zn	52,4a	5,58abc	268a	124,1a	27,5ab	22,3c	1,494b	8,59b
G4	Ø	33,4bc	4,2bc	139b	81,2bc	35,6ab	43,8ab	1,459b	7,07b
	Zn	23,6c	4,0c	93b	78,0bc	28,7ab	36,8ab	1,584b	4,42b

SP – stems per plant, IS – inflorescences per stem, IP – inflorescences per plant, FI – flowers per inflorescence, SI – seeds per inflorescence, TSW – thousand seed weight (g), F - fertility (%), SYP – seed yield per plant (g); Values followed by different small letters within columns are significantly different ($P<0.05$) according to the LSD test.

The average seed yield in this experiment was 9.3 g per plant. Herrmann et al. (2006) state that the average seed yield per plant in two genotypes of red clover was 5.72 g, with the variation interval of 0.71-11.31g. According to Vasiljević et al. (2000), the coefficients of genetic correlation show that seed yield per plant mostly depended on the number of seeds per inflorescence, the number of flowers per inflorescence and the number of productive stems per plant. The authors also claim that there were significant positive genetic correlations between the number of inflorescences and the number of internodes per stem and green matter yield, as confirmed by Steiner and Alderman (2003). Foliar application of zinc in this experiment had a significant positive effect on seed yield per plant only in G1. In the control variant, the genotypes did not differ significantly in terms of seed yield per plant, while in the treatment with foliar application of zinc, significantly higher seed yield per plant was recorded in G1 compared to all other genotypes. A possible reason for the different response of genotypes to foliar treatments with zinc in terms of seed yield and seed yield components may be the different proportion of leaves at the time of treatment (author's observations). According to numerous studies, zinc has a great influence on the basic life processes of plants, such as: metabolism and nitrogen intake; photosynthesis and chlorophyll synthesis, carbonic anhydrase activity; resistance to abiotic and biotic stresses; protection against oxidative damage (Potarzicki and Grzebisz, 2009; Cakmak, 2008; Mousavi, 2011). Zinc enters into the composition of over 300 plant enzymes (Ramezani et al., 2016). Due to the mentioned unsubstituted effects, zinc plays a crucial role in plant growth and development, so it can greatly influence the yield components and seed yield of cultivated plants (Derakhshani et al., 2011; Farnia and Khodabandehloo, 2015). According to the results of Ramezani et al. (2016) foliar application of zinc in alfalfa in a concentration of 0.3% led to an increase in its concentration in plants, which led to an increase in dry matter yield. Farnia and Khodabandehloo (2015) state that the application of the chelated form of zinc in maize in the phase of 4-6 leaves affected the increase in the number of seeds per cob and the thousand seed weight, which led to an increase in seed yield and harvest index. Poblaciones et al. (2017) indicate that zinc as a heavy metal antagonist, foliar application in legumes, may reduce the uptake of heavy metals from contaminated soils. The authors indicate that foliar application of 0.25% w/v $ZnSO_4 7H_2O$ decreased stem Cd concentration in *L. rigidum* and *T. subterraneum* grown in Cd-contaminated soil and ameliorated the adverse effects of Cd exposure on root growth, mainly in *T. subterraneum*.

CONCLUSION

Foliar application of zinc had a positive effect on the number of stems per plant, the number of inflorescences per stem, the number of inflorescences per plant and the number of flowers per inflorescence in certain red clover genotypes. The negative impact of foliar application of zinc was observed on the thousand seed weight and flower fertility in red clover genotypes originating from Bulgaria. A significant increase in seed yield per plant under the influence of foliar application of zinc was recorded only in the variety K-39, which otherwise had the highest yield. The analyzed genotypes of red clover from natural populations had significantly lower seed yield in the treatment with zinc compared to the variety K-39, but they had higher values for certain yield components. As such, they can be used in selection work.

ACKNOWLEDGEMENTS

The research was financed by the Ministry of Education, Science and Technological Development, Republic of Serbia, project ref. number 451-03-9/2021-14.

REFERENCES

Ali L., Ali M., Mohyuddin Q. (2011). Effect of foliar application of zinc and boron on seed cotton yield and economics in cotton-wheat cropping pattern. Journal of Agricultural Research, 49(2): 173-180.

Cakmak I. (2008). Enrichment of cereal grains with zinc: Agronomic or genetic biofortification? Plant and Soil, 302: 1-17.

Derakhshani Z., Hassani A., Sadaghiani M.H.R., Hassanpouraghdam M.B., Khalifani H.B., Dalkani M. (2011). Effect of Zinc Application on Growth and Some Biochemical Characteristics of Costmary (*Chrysanthemum balsamita* L.). Communications in Soil Science and Plant Analysis, 42: 2493–2503.

Efe L., Yarpuz E. (2011). The effect of zinc application methods on seed cotton yield, lint and seed quality of cotton (*Gossypium hirsutum* L.) in east

Mediterranean region of Turkey. African Journal of Biotechnology, 10: 8782-8789.

Farnia A., Khodabandehloo S. (2015). Changes in Yield and its Components of Maize (*Zea mays* L.) to Foliar Application of Zinc Nutrient and Mycorrhiza under Water Stress Condition. International Journal of Life Sciences, 9(5): 75-80.

Galavi M., Yosefi K., Ramrodi M., Mousavi S.R. (2011). Effect of bio-phosphate and chemical phosphorus fertilizer accompanied with foliar application of micronutrients on yield, quality and phosphorus and zinc concentration of maize. Journal of Agricultural Science, 3(4): 22-29.

Helgadottir A. (1996). Legumes in Icelandic agriculture. Grassland and land use systems. Grassland science in Europe, 1: 741-745.

Herrmann D., Boller B., Studer B., Widmer F., Kolliker R. (2006). QTL analysis of seed yield components in red clover (*Trifolium pratense* L.), Theoretical and applied genetics, 112 (3): 536-545.

Jevtić G., Radović J., Lugić Z., Sokolović D., Valić T. (2007). Influence of honey bee (*Aphis mellifera* L.) and sugar syrup on alfalfa and red clover seed yield. Proceedings, IX Symposium of Forage Plants of the Republic of Serbia, 44(1): 99-106.

Kadović R., Knežević M. (2002). Heavy metals in forest ecosystems of Serbia, University of Belgrade, Faculty of Forestry, Ministry of Natural Resources and Environmental Protection of the Republic of Serbia, Belgrade.

Kinaci G., Kinaci E. (2005). Effect of zinc application on quality traits of barley in semi arid zones of Turkey. Plant, Soil and Environment, 51(7): 328–334.

McBride B.M., Blasiak J.J. (1979). Zinc and Copper Solubility as a Function of pH in an Acid Soil. Soil Science Society America Journal, 43: 866-870.

Mousavi R.S., Galavi M., Rezaei M. (2013). Zinc (Zn) Importance for Crop Production – A Review. International journal of Agronomy and Plant Production, 4(1): 64-68.

Mousavi S.R. (2011). Zinc in crop production and interaction with phosphorus. Australian Journal of Basic and Applied Sciences. 5: 1503-1509.

Mousavi S.R., Galavi M., Ahmadvand G. (2007). Effect of zinc and manganese foliar application on yield, quality and enrichment on potato (*Solanum tuberosum* L.). Asian Journal of Plant Sciences, 6: 1256-1260.

Outten E., O'Halloran T.V. (2001). Femtomolar sensitivity of metalloregulatory protein controlling Zn homeostasis. Science, 292: 2488-2492.

Pandey N., Pathak G.C., Sharma C.P. (2006). Zinc is critically required for pollen function and fertilisation in lentil. Journal of Trace Elements in Medicine and Biology, 20: 89-96.

Pedler J.F., Parker D.R., Crowley D.E. (2000). Zinc Deficiency-induced phytosiderophore release by the Triticaceae is not consistently expressed in solution culture. Planta, 211:120-126.

Poblaciones J.M., Damon P., Rengel Z. (2017). Foliar zinc biofortification effects in *Lolium rigidum* and *Trifolium subterraneum* grown in cadmium-contaminated soil. PLoS ONE, 12(9): 1-11.

Potarzycki J., Grzebisz W. (2009). Effect of zinc foliar application on grain yield of maize and its yielding components. Plant, Soil and Environment, 55(12): 519–527.

Ramezani A., Sajedi A.N., Yarahmadi M.H. (2016). The Effect of Foliar application of Iron, Zinc, and Selenium on Nutrient Uptake and Yield of Alfalfa. International Research Journal of Applied and Basic Sciences, 10(5): 557-562.

SPSS.Inc. 1995.STATISTICA for Windows (Computer Program Manual). Tulsa. OK.

Steiner J.J., Alderman C.S. (2003). Red clover seed production: VI. Effect and Economics of Soil pH Adjusted by Lime Application, Crop Science, 43: 624-630.

Steiner J.J., Leffel J.A., Gingrich G., Aldrich-Markham S. (1995). Red clover seed production: III Effect of herbage removal time under varied environments. Crop Science, 35: 1667-1675.

Taylor N.M., Smith R.R. (1980). Red clover breeding and genetics. Advances in Agronomy, 31: 125-154.

Vasiljević S., Šurlan-Momirović G., Katić S., Mihailović V., Lukić D., Živanović T. (2000). Relationships between green forage and seed yield components in genotypes of red clover (*Trifolium pratense* L.), Genetika, 32(1): 37-43.

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.

Xi-Wen Y., Xiao-Hong L., Xin-Chun T., William G.J., Yu-Xian C. (2011). Foliar zinc fertilization improves the zinc nutritional value of wheat (*Triticum aestivum* L.) grain. African Journal of Biotechnology, 10: 14778-14785