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Boron application in red clover (*Trifolium pratense* L.) seed production

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Abstract

A field trial with four red clover cultivars was established on acid soil in order to evaluate the effect of foliar boron application on seed yield. The crop received foliar boron treatment during the second growth of the second year at two applications. Although seed yield showed a significant increase in boron-treated plants in 2011 compared with control (26.0%), its relative increase was far higher in 2010 (43.2%), which had increased total rainfall amounts during flowering. Sufficient level of boron supply to red clover plants for seed production has a remarkably positive effect under conditions hampering pollination and fertilisation.

Keywords

boron • red clover • seed yield • yield components

Introduction

In Southeastern Europe, red clover (*Trifolium pratense* L.) seed crops are often grown on acid soils where certain macro- and micronutrients are less available to plants (Dear and Lipsett, 1987).

As a micronutrient, boron (B) is vital to many cellular processes in plants (Hu and Brown, 1994; Matoh *et al.*, 2000; Dos Santos *et al.*, 2004). Sufficient B levels also enhance nitrogenase activity and nitrogen fixation by *Rhizobium*, thereby facilitating the growth of legume plants (Blevins and Lukaszewski, 1998).

Specifically, insufficient B supply has an unfavourable effect on the growth and development of generative organs of many plants. B deficiency generally leads to precocious flowering (Hanson, Chaplin and Breen, 1985) and poor quality fruit (Gupta, 1993). Positive effects of sufficient B levels include an increased number of fertile flowers (Dear and Lipsett, 1987; Noppakoonwong *et al.*, 1997), higher pollen viability (Ylstra *et al.*, 1992) and improved seed and fruit growth (Rashid, Rafique and Bughio, 1994).

B is uptaken from the soil mostly as undissociated boric acid (Hening and Patrick, 1997). B uptake in acid soils is adversely affected by a high concentration of aluminium ions (Yang and Zhang, 1998; Matsumoto, 2000; Seguel *et al.*, 2012). Therefore, foliar B application in acid soils would be an efficient strategy to establish optimal B status in plants compared with its supplementation via soil. The objective of the present study was to evaluate seed yield and yield components in red clover cultivars when supplied with foliar application of boron, a micronutrient not readily available to plants in acid soils.

Materials and methods

Soil properties

A field experiment was set up at a trial field of the Veterinary Extension Service in Čačak (43°54'39.06" N, 20°19'10.21" E, 246 m a.s.l.) in 2009–2011. The trial was established on a fluvisol (IUSS Working Group WRB, 2014), pH_{H2O} 4.8, with 3.18% organic matter, 0% $CaCO_3$, 22.08mg P $100g^{-1}$ soil and 30.0mg K $100g^{-1}$ soil (Gupta 2008). The preceeding crop was natural meadow. In autumn, prior to seeding, 45 kg ha^{-1} N, 45 kg ha^{-1} P_2O_5 and 45 kg ha^{-1} K_2O were incorporated into the soil by primary tillage.

Weather conditions

Data on mean monthly temperatures and rainfall were recorded throughout the experiment at a weather station located near the experiment. The mean annual air temperature in 2010, 2011 and long-time period (1992–2002) was 12.57, 12.37 and 11.97ºC, respectively. Monthly rainfall (Figure 1) showed large variations during the growing season (April–September).

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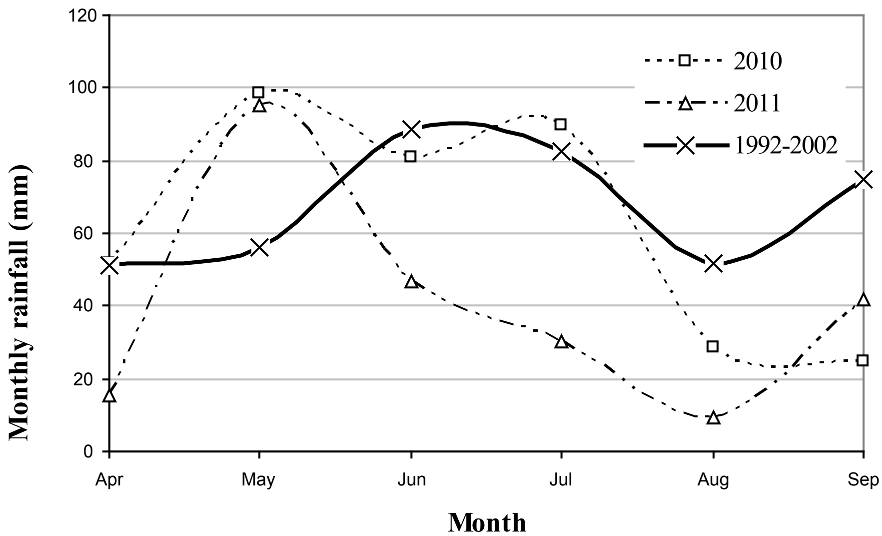

Figure 1. Monthly rainfall distribution for experimental years and 10-year (1992–2002) average

Experimental design

The experiment, which included four diploid red clover cultivars and two treatments with boron (control, foliar B), was conducted as a randomised block design with four replications, with a plot size of 5 m^2 (5 x 1 m). Red clover cvs. K-17, K-39 (Institute of Forage Crops, Kruševac, Serbia), Una (Institute of Field and Vegetable Crops Novi Sad, Serbia) and Viola (a Polish cultivar) were planted at 20 cm row spacing at a seed rate of 18 kg ha^{-1}. The cultivars were sown on 3 April 2009 and 27 March 2010. The crop was subjected to foliar treatment with B (Bor-feed, Haifa, Izrael, with 11% of boron) at a concentration of 0.1% and water rate of 1000 l ha^{-1} (0.11 kg ha^{-} B) at two applications, that is, the first during intensive growth (June 5), the second before the onset of flowering (June 25). Weeds were controlled mechanically. No irrigation was employed.

Measurements

Seed yield and yield components were determined from the second cut in the second year of cultivation (in 2010 and 2011 for establishments in 2009 and 2010, respectively). In the field, the following yield components were determined at flowering time: stem number m^{-2} and inflorescence number m^{-2} (by counting within an area of 0.2 m^2 per plot), and inflorescence number per stem (by counting in a sample of 10 randomly selected middle-row stems per plot). A sample of 10 inflorescences was taken from each plot at the time of full maturity and the following traits were determined: flower number per inflorescence, seed number per inflorescence and thousand seed weight (based on the weight of 5 x 100 seeds extracted from the same sample). Fertility was calculated from the number of flowers and seeds per inflorescence and expressed in percentages. The actual seed yield was determined on the basis of yield components (inflorescences per unit area, seeds per inflorescence, thousand seed weight) and converted to seed yield in kg ha^{-1}.

The results were subjected to analysis of variance using SPSS (SPSS, 1995). Differences between means were tested by LSD test. When the interactions were significant, the main effects were not discussed. To satisfy the requirement of normality, the data for stem number m^{-2}, inflorescence number m^{-2}, flower number per inflorescence, seed number per inflorescence are transformed before analysis of variance using the form $\sqrt{x}$ or $\sqrt{x} + 1$ for inflorescence number per stem.

Results and discussion

The role of B in nitrogen fixation in legumes lies in its positive effect on nitrogenase activity (Blevins and Lukaszewski, 1998) and growth of *Rhizobium* bacteria (Loomis and Durst, 1992). In the present study, stem number m^{-2} after B treatment was significantly higher only in cvs. K-17 and Viola, compared with the control in both years (cultivar/foliar treatment interaction) (Table 1), which was due to their relatively higher leaf percentage during the treatment (the authors' note). Regardless of foliar B application, significantly higher stem number m^{-2} in 2011 was produced in cv. K-17 compared with cvs. K-39 and Viola (year/cultivar interaction), which was likely indicative of its higher tolerance to soil moisture deficiency.

B generally exhibits a markedly higher effect on the development of generative plant organs than on vegetative

Table 1. Seed yield components: SNM, stem number m^{-2};, INM, inflorescence number m^{-2};, INS, inflorescence number per stem; FNI, flower number per inflorescence; SNI, seed number per inflorescence; F (%), fertility; TSW (g), thousand seed weight and SY, seed yield (kg ha^{-1}) in red clover cultivars as affected by foliar treatment with boron (control - 0).

		SNM	INM	INS	FNI	SNI	F	TSW	SY
Year	2010	338.3	568b	1.66b	67.7b	34.2b	50.8	1.38	268b
	2011	350.9	853a	2.43a	99.6a	52.4a	53.7	1.63	621a
Cultivar	K-39	339.1ab	732ab	2.17	83.5ab	43.2b	51.0b	1.41	453a
	K-17	358.8a	761a	2.09	83.1ab	41.8b	51.0b	1.48	497a
	Una	357.5a	740ab	2.06	94.7a	52.8a	58.6a	1.39	561a
	Viola	321.1b	609b	1.87	73.2b	35.5c	48.4b	1.75	267b
Foliar t.	O	331.7b	675	2.02	76.9b	40.4b	51.4	1.62	385b
	B	357.5a	746	2.08	90.3a	46.2a	53.1	1.39	504a
2010	K-39	347.5abc	657cde	1.89	68.2cd	33.0	48.1b	1.44	303cd
	K-17	338.1c	613de	1.77	69.8cd	31.1	47.8b	1.54	289cd
	Una	345.0bc	534e	1.55	72.4cd	42.9	58.9a	1.40	310cd
	Viola	322.5c	467e	1.45	60.3d	29.8	48.3b	1.14	169d
2011	K-39	330.6c	806abc	2.45	98.9ab	53.4	54.0ab	1.38	602b
	K-17	379.4a	910ab	2.41	96.4ab	52.5	54.1ab	1.42	704ab
	Una	370.0ab	945a	2.56	117.0a	62.7	58.3ab	1.38	811a
	Viola	323.8bc	751bcd	2.30	86.1bc	40.9	48.5ab	2.35	364c
2010	O	330.3b	550	1.64	61.1	30.5c	48.8c	1.35	220c
	B	346.2ab	586	1.69	74.2	37.9b	52.8b	1.41	315c
2011	O	333.1b	799	2.39	92.8	50.2a	54.1a	1.89	549b
	B	368.8a	906	2.47	106.4	54.5a	53.8a	1.37	692a
K-39	O	323.8cd	682	2.12	80.3bc	40.1	49.0	1.43	398
	B	354.4abc	780	2.21	86.8b	46.3	53.1	1.39	508
K-17	O	340.6bc	710	2.04	83.9bc	39.5	45.9	1.45	424
	B	376.9a	812	2.15	82.4bc	44.1	56.1	1.52	570
Una	O	356.9ab	767	2.14	79.9bc	49.6	57.7	1.32	508
	B	358.1ab	712	1.97	109.4a	55.9	59.5	1.45	614
Viola	O	305.6d	539	1.76	63.7c	32.2	47.2	2.28	209
	B	340.6bc	679	1.99	82.7bc	38.5	49.5	1.21	324

Values followed by different small letters within columns are significantly different (P < 0.05) according to the LSD test.

organs (Bernie and Longbin, 1997). In the present study, foliar B application had no significant effect on inflorescence number m^{-2} and inflorescence number per stem in all cultivars in both years. Wilczek and Ćwintal (2008) found no significant effect of foliar B application in red clover during the bud formation stage on inflorescence number per stem. Schon and Blevins (1990) reported significantly more branches per plant and pods per branch in soybean as the result of B application at the onset of intensive growth. These results suggest that the effect of foliar-applied B on certain yield components is dependent on plant development stage during the treatment.

B application significantly increased the number of flowers per inflorescence in cv. Una in both years (cultivar/foliar application interaction), although a positive response was observed in all cultivars except cvs. K-17. B deficiency inhibits or retards flower development, particularly in plants developing flowers in compact terminal inflorescences (Dell and Huang, 1997).

As the result of foliar B application, seed number per inflorescence significantly increased in all cultivars in 2010. B enhances pollen viability and germination and ensures the increased presence of insect pollinators in red clover (Wilczek and Ćwintal, 2008). Regardless of foliar B application, seed number per inflorescence was significantly higher in cv. Una compared with the other cultivars in both years (42.9 and 62.7, respectively). Wilczek and Ćwintal (2008) reported a range of 61 to 74 for seed number per inflorescence in cv. Parada. In the present study, all cultivars had a relatively low number of seeds per inflorescence, which was attributable to high plant density and less favourable environmental conditions.

A significant increase in fertility in all cultivars was obtained with B treatment in 2010. Foliar B application and declining rainfall during flowering (the beginning of July, Figure 1) induced a significant increase in flower fertility in red clover (Wilczek and Ćwintal, 2008). The results obtained suggest that the positive effect of foliar B application on pollen grain quality, pollination and fertilisation is remarkably high under increased rainfall during clover flowering. Lewis (1980), Dell and Huang (1997) and Dordas (2006) also indicated positive effects of B on pollination, fertilisation and seed and fruit growth.

Foliar-applied B induced a significant increase in seed yield in all cultivars in 2011 (year/foliar application interaction). However, relative yield increase was far higher in 2010 (43.2%), although not significant (high variability over replications, CV = 26%), compared with 2011 (26.0%). This indicating that B supply in red clover comes to the forefront during conditions hampering pollination and fertilisation. Positive effects of foliar B application on seed yield in red clover were also reported by Lewis (1980), Wilczek and Ćwintal (2008) as well as by Schon and Blevins (1990) in soybean and Dordas (2006) in alfalfa. Favourable weather in the first part of the growing season and lower rainfall during flowering in 2011 contributed to increasing total seed yield compared with 2010, which is in agreement with the findings of Gorski and Bawolski (1989), and Wilczek and Ćwintal (2008).

In this research, foliar B application had a positive effect on yield components and seed yield in all cultivars. A sufficient level of B supply to red clover plants for seed production has a remarkably positive effect, especially under conditions hampering pollination and fertilisation. The results suggest that foliar B application in red clover can increase seed production on acid soils, particularly when less favourable weather conditions occur during flowering.

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