Biosis: Biological Systems (2020) 1(2): 60-64
https://doi.org/10.37819/biosis.001.02.0056

ORIGINAL RESEARCH

The Effect of Foliar Boron Application on Seed Production of Alfalfa (*Medicago sativa* L.)

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ABSTRACT

Boron (B) is an essential micronutrient for plant reproductive growth and seed setting. A better understanding of the reasonable application rate of B could provide guidelines for improving seed yield and quality. In this study, we used five B concentrations (0, 400, 800, 1200, and 1600 mg B L^{-1}) to study the effect of foliar application of B on seed yield and quality of alfalfa (*Medicago sativa* L.) in northern China. Our results indicated that foliar B application increased the pollen number, pollen viability, and dry weight and B concentration of alfalfa reproductive organs. The effect of B on alfalfa seed yield can be attributed to affecting the number of inflorescence and the seeds per pod. Foliar application with 800 mg B L^{-1} made quantitative and qualitative improvements in seed yield and quality of alfalfa. These results help to explain the positive effects of B on alfalfa seed production.

ARTICLE HISTORY
Received 11 May 2020
Revised 31 May 2020
Accepted 31 June 2020

KEYWORDS
Alfalfa
Boron
Foliar application
Seed yield, Seed quality

Introduction

Alfalfa (*Medicago sativa* L.) is an important forage crop worldwide because of its strong adaptability, rich nutrition, and high feeding value (Dordas, 2006). However, seed production technology is backward, which has been neglected for a long time in China; worryingly, global seed yield is only approximately 4% of the theoretical seed yield (Wang et al., 2007). Such low yield cannot provide the needs of large-scale cultivation in this region. Seed yield is a complex trait that composed of several individual yield components (Dordas, 2006). Therefore, there are many factors that influence seed yield and quality under field conditions (Wang et al., 2007).

Boron (B) is an essential element required for maximizing seed yield and yield components in alfalfa (*Medicago sativa* L.) (Chen et al., 2017). B plays a vital role in pollen germination, flower development, nitrogen metabolism, carbohydrate biosynthesis, and many other processes during the reproductive growth phase of alfalfa plants (Abreu et al., 2012; Terzic et al., 2012). There is a much higher demand for B during the reproductive growth (flowering and seed set) of alfalfa even if the level of this element is adequate in soil (Dordas, 2006). Previous reports show that an adequate supply of B is critical for flower development and seed formation in alfalfa (Abreu et al., 2012; Terzic et al., 2012). However, seed growers have often neglected the rate of B application during the alfalfa seed production. Although an increasing body of studies has focused on the effects of B on the yield and quality of alfalfa seeds, however, B can have positive or negative effects on alfalfa seed yield depending on application methods, B concentration, soil type, and alfalfa cultivars (Dordas, 2006; Chen et al., 2017).

As calcareous soils with a low B availability are widely distributed in northern China. Alfafa is planted here over large scale of alfalfa, which is easy to cause boron deficiency during the alfalfa seed production (Liu et al., 1980). Therefore, it is necessary to study the appropriate rate of B fertilizer for this region. In this study, we aimed to investigate the effects of different B treatments on alfalfa seed yield, yield components, and seed quality, so as to determine the appropriate rate of B fertilizer for alfalfa seed production.

Materials and methods

B treatments

The alfalfa cultivar 'Gongnong No.1' was grown in the experimental field of the Chifeng Academy of Agricultural and Animal Sciences (longitude 118.51°E; latitude 42.17°N) in 2014. The size of an individual plot was 3.8×5.5 m, and each plot contained six rows with a space of 60 cm between each row. Soil was sampled at a depth of 0–30 cm prior to planting, and analyzed for B content and pH. Plants were grown in a randomized complete block design with four replicates and subjected to five B treatments: (0, 400, 800, 1200, 1600 mg B L^{-1}), where B was foliar sprayed at the first flowering stage on the 6th of June 2015. B was applied in the form of sodium tetraborate containing 11.34% of B ($Na_2B_4O_7 \cdot 10H_2O$, Borax, Tianjin, China). The foliar spray was applied with a Knapsack electric sprayer (SX-MD16E-2L, SeeSa, Zhejiang, China) at 500 kPa pressure and applying 750 L of water ha^{-1}.

Determination of pollen-producing capacity and pollen viability

After 7 days of B application, flowers opening at the same time (fully opened but the keels still closed) were harvested randomly from each treatment and used for microscopic analysis. To determine the pollen-producing capacity of alfalfa plants, mature anthers was removed from 10 flowers per plot and incubated in 1 mL of 2.5% cellulose for 24 h. The sample was then diluted in 9 mL of 2.5% sucrose, and the number of pollen grains was counted under a microscope (XSP-300, China). To determine pollen viability, pollen grains harvested from 10 flowers were pooled and placed on culture medium supplemented with 10% sucrose, 0.02% B, and 1% agar. The samples were incubated at 25 ℃ for 1.5 h and pollen tube germination was observed under a microscope (Pandey & Gupta, 2013). Pollen grains were considered germinated when the pollen tube length was larger than pollen grain diameter.

Determination of dry weight and B concentration of alfalfa reproductive organs

In order to determine the dry weight and B concentration, tem plants were harvested from each plot (thirty plants per treatment) at the flowering stage and seed maturity stage, and different reproductive organs (flowers, and seeds) were separated. All flowers and seeds were dried in an oven at 70 ℃ until a constant weight was achieved, and then weighed to determine the dry weight. Dried samples were ground into a fine powder, dry-ashed at 500 ℃ for 4 h, and suspended in 100 mM HCl. B concentration was then determined according to the Curcumin-oxalic acid method (John et al., 1975) using a spectrophotometer (Hitachi UV-3100 UV/VIS; TECHCOMP, Shanghai, China).

Determination of seed yield and yield components

Plants were tagged before the foliar application of Borax, and seed yield components were determined using ten plants per plot. The number of twigs per square meter, racemes per plant, and flowers per raceme were counted at 7 days after foliar application. When 50–60% of the plants entered the pod stage, the number of pods per raceme and seeds per pod were measured. Seed yield was determined when 70–80% of the pods turned a deep yellow color. Seed yield was gained from three 1.8 m^2 sampling unit (three row with 1 m length) from each plot.

Determination of seed quality

To determine seed quality, mature seeds obtained from different B treatments were tested according to the rules of ISTA (2015). Four replicates of 100 seeds obtained from each treatment were germinated on filter paper moistened with distilled water in a germination incubator (GXZ-380B, China) at 20 ℃ under 8 h light/16 h dark cycle. The number of normal seedlings, abnormal seedlings, hard seeds, and dead seeds were counted after 10 d. Seed germination percentage was calculated according to the number of normal seedlings and hard seeds.

Statistical analysis

All data were analyzed using a one-way ANOVA and SPSS 21.0. Between treatment effects were assessed using Tukey's honestly significant difference test. All analyses were tested at $P <$ 0.05.

Results and discussion

Effect of foliar B application on the pollen number and pollen viability in alfalfa

The pollen number of alfalfa was significantly increased ($P < 0.05$) at 800 mg B L^{-1} compared with the control treatment, but significantly reduced ($P < 0.05$) at 1600 mg B L^{-1} compared with the 800 mg B L^{-1} treatment (Table 1). This suggested that higher concentration of B had negative effects in pollen germination. In addition, pollen viability of foliar B applied plants increased significantly ($P < 0.05$) over that in 0 mg B L^{-1} plants. The pollen number and viability highest increased at 800 mg B L^{-1} by 63.75% and 165.65%, respectively, compared with the control treatment. These findings suggest that foliar supply of B in appropriate doses has a beneficial effect on pollen germination of alfalfa in this region. The increase in pollen number and viability after B application might be due to B affecting H+-ATPase activity, which initiates pollen germination and tube growth (Pandey and Gupta, 2013). Studies have shown that B also involved in the delivery of carbohydrates necessary for starch accumulation in anther, which may have also contributed to the pollen maturation (Ruuhola et al., 2011).

Table 1. Effect of B treatment on the pollen number, pollen viability, dry weight, and B content in the reproductive organs of Alfalfa.

Treatments (mg B L^{-1})	Pollens per flower	Pollen viability (%)	Dry weight (g·plant^{-1})		B content (mg·kg^{-1})	
			Flowers	Seeds	Flowers	Seeds
0	3200±178.89cd	20.67±2.38b	0.50±0.03b	1.44±0.05b	34.28±1.37c	23.85±0.60b
400	3880±332.27bc	54.56±9.62a	0.56±0.04b	1.60±0.03b	42.33±0.61b	26.95±0.74ab
800	5240±307.57a	54.91±11.52a	0.73±0.04ab	1.86±0.03a	54.28±1.59a	29.85±0.68a
1200	4640±172.05ab	54.20±3.28a	0.80±0.04a	1.84±0.03a	52.33±0.73a	25.95±1.05ab
1600	2680±149.67d	53.42±6.11a	0.81±0.04a	1.41±0.05b	55.43±1.49a	25.89±0.71ab
F	18.677	7.596	7.901	17.894	33.100	4.807
P	<0.001	<0.001	0.004	<0.001	<0.001	0.020

Note: Different letters within a column indicate treatment effects at $P < 0.05$ (Tukey honestsignificant difference test).

Effect of foliar B application on dry weight and B concentration of alfalfa reproductive organs

Foliar B sprayed plants showed higher dry weight and B concentration than those grown under control conditions (Table 1). 1200 mg B $L^{-1}$and 1600 mg B L^{-1} treatments significantly increased ($P < 0.05$) the flower dry weight, whereas 800 mg B L^{-1} and 1200 mg B L^{-1}treatment significantly increased ($P < 0.05$) the seed dry weight compared with the control treatment. In addition, all B treatments significantly increased ($P < 0.05$) the B concentration in flowers, however, only 800 mg B L^{-1}treatment significantly increased ($P <$ 0.05) the concentration of B in seeds. It is commonly accepted that foliar B application can increase fruit and seed set in many plants more effectively than that of soil B application, because flowers and seeds might not be able to take up B directly from the soil like leaves (Brown et al., 2002). Foliar application of B not only provides way to apply at specific growth stage, but also correct its deficiency. Therefore, we speculated that the positive effect of foliar B application on alfalfa seed production is that B is transported to the developed flowers, which improving the vitality and fertility of pollen and promoting the flower development (Zheng et al., 2019).

Effect of foliar B application on seed yield and yield components of alfalfa

Foliar B application significantly increased ($P <$ 0.05) the seed yield of alfalfa plants, but did not induce significant changes ($P > 0.05$) in seed yield components except racemes per square meter (Table 2). These findings suggest that the increase in the number of inflorescence improves the potential for increased seed yield. Another reason for the increase in seed yield is attributed to the fact that the number of flowers per raceme of B treated plants was slightly smaller, which could increase the number of seeds per pod and also increase the pod setting rate. Similar results were reported in previous study (Du et al, 2009). Pod abortion is one of the major factors that affecting seed yield in alfalfa seed production (Dordas, 2006). The higher number of flowers per raceme and lower pod setting rate in the control treatment in our study confirmed these findings, indicating that pod abortion occurred under B deficiency conditions.

B of 800 mg B L^{-1} was effective in most cases in increasing the seed yield compared with the control treatment. However, higher rates of B reduced the seed yield and yield components, indicating that higher B concentrations have negative effects on alfalfa seed production. This may be because the rage between optimal and toxic B concentrations is very narrow (Gupta et al., 1985). Therefore, from the present data we can conclude that foliar B application of 800 mg B L^{-1} is beneficial for alfalfa seed production in study area.

Table 2. Effect of B treatment on the seed yield and seed yield components of Alfalfa.

Treatments (mg B L^{-1})	Racemes per m^2	Flowers per raceme	Pods per raceme	Seeds per pod	Pod setting rate (%)	Seed yield ($g \cdot m^{-2}$)
0	4078±53.67d	20.21±0.54ab	11.7±0.54a	7.24±0.49a	57.8±1.22ab	19.28±1.05c
400	6051±93.61b	21.73±0.50a	10.07±0.34a	7.03±0.18a	46.37±1.35b	35.47±0.51ab
800	7237±24.46a	19.96±0.47ab	11.96±0.27a	7.52±0.37a	60.12±2.68a	39.40±1.44a
1200	5596±175.35b	19.03±0.49ab	11.22±0.46a	8.00±0.31a	59.2±2.03a	31.91±0.62b
1600	4937±86.73c	18.34±0.28b	10.86±0.37a	7.44±0.11a	59.22±2.16a	29.47±1.93b
F	83.758	4.605	1.989	0.767	5.147	22.93
P	<0.001	0.023	0.172	0.57	0.016	<0.001

Note: Different letters within a column indicate treatment effects at $P < 0.05$ (Tukey significant difference test).

Effect of foliar B application on seed quality of alfalfa

Compared with the control, B treatment did not induce the significant changes ($P > 0.05$) in alfalfa seed germination percentage (Table 3). These results were disagreed the findings of previous studie (Dordas, 2006). 800 mg B L^{-1} treatment was significantly increased ($P < 0.05$) the germination potential and reduced ($P < 0.05$) the percentage of hard seeds, indicating that seeds with adequate B supply can germinate and produce seedlings faster and with better ability to grow.

The results obtained here suggest that foliar B application can improve the seed yield and yield components of alfalfa grown for seed production. The 800 mg B L^{-1} treatment was found to have a positive response in most characteristics. The results presented here provide some clear guidelines for improving seed yield and quality in studied regions.

Table 3. Effect of B treatments on the seed quality of Alfalfa.

Treatments (mg B L^{-1})	Germination percentage (%)	Germination potential (%)	Hard seeds (%)	Abnormal seedlings (%)	Dead seeds (%)
0	85.92±0.17a	37.00±2.64b	39.58±0.98a	6.42±0.28ab	7.67±0.13ab
400	86.25±2.33a	38.83±2.48b	41.42±1.01a	5.75±0.11b	8.00±0.22a
800	86.00±1.07a	53.83±2.44a	29.08±2.68b	7.08±0.23ab	6.92±0.17b
1200	85.50±0.40a	37.85±1.29b	37.67±0.93ab	7.00±0.40ab	7.50±0.19ab
1600	85.33±0.81a	37.64±1.25b	42.08±1.66a	7.50±0.19a	7.08±0.17ab
F	0.057	6.948	6.468	4.006	3.551
P	0.993	0.006	0.008	0.034	0.047

Note: Different letters within a column indicate treatment effects at $P < 0.05$ (Tukey significant difference test).

Acknowledgements

We thank Ms. Qingwei Liang, Ms Xiufang Yang, and Miss Risu Na for providing technical support for field research and plant sampling.

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