RESEARCH ARTICLE Legume Research- An International Journal, Volume 43 Issue 3: 415-420 (June 2020)

Effect of Boron Priming on the Seed Vigour in Different Varieties of Alfalfa (*Medicago sativa* L.)

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10.18805/LR-484

ABSTRACT

This experiment was designed to determine the influence of boron priming on seed vigour in thirteen varieties of alfalfa (*Medicago sativa* L.). Alfalfa seeds were primed with 1.8% (W/V) concentration of borax solution for 0, 3, 6, 9, 12 and 24 h at 20°C. The results showed that the original vigour of alfalfa seeds was disparate in thirteen varieties and there were highly significant ($P<0.01$) differences observed in both varieties and priming time and their interactions on the vigour of the seeds. Alfalfa seeds of WL525HQ and WL656HQ might be more sensitive to boron toxicity, but seeds of WL298HQ, WL343HQ, WL354HQ, WL903 and Pianguan were insensitive to boron toxicity. Thus, it is necessary to carefully select appropriate varieties of alfalfa seeds for the application of boron priming.

Key words: Alfalfa, Boron, Germination, Seed priming, Seed vigour.

INTRODUCTION

Alfalfa (*Medicago sativa* L.) has more excellent features comparing with other forage crops, *e.g.* high yield, rapid regeneration, high adaptability, rich nutrition and low production cost (Zhang *et al*., 2019). Therefore, it is considered the most important forage crop and is extensively cultivated primarily for hay, silage and pasture in animal feeding around the world (Hawkins and Yu, 2018; Yang *et al*., 2019). Furthermore, alfalfa can also be used to improve soil fertility as an important green manure crops (Zhang *et al*., 2017a). Currently, the cultivation of alfalfa is increasing rapidly in the world, especially in arid and semi-arid areas (Huang *et al*., 2018). Hence, the quantity and quality of alfalfa seeds should be improved correspondingly. However, the amount of alfalfa seed fields is not growing at a sufficient rate and the yield of alfalfa seeds is still relatively low (Zhang *et al*., 2017b), this seriously restricts the splendid development of alfalfa industry around the world. Consequently, how to improve alfalfa seed yield has become a world problem to promote the rapid development of alfalfa industry.

Boron is an important micronutrient for vascular plants. In recent years, it has been used more widely in order to increase grain yield in crop production (Parry *et al*., 2016; Iqbal *et al*., 2017). Likewise, alfalfa seed yield can be effectively improved using foliar application of boron (Du *et al*., 2009). Nevertheless, there are still few studies on the application of boron to improve alfalfa seed yield and it is especially scarce for research on the optimal application methods. Compared with soil application and foliar spray, seed priming has been emerged as an attractive and easy physiological strategy in micronutrients application (Farooq *et al*., 2012; Arun *et al*., 2017). Currently, it is necessary to discover and optimize new priming agents in order to satisfy the cheaper, exercisable and easily accessible requirements of farmers (Hussain *et al*., 2018). Seed priming with boron has been shown to be effective in encouraging seedling emergence, growth and grain yield during crop production (Iqbal *et al*., 2017). Moreover, our previous study also found that appropriate priming with boron can improve the seed vigour of alfalfa (Xia *et al*., 2019). Nonetheless, the understanding remains poor in response of boron priming on seed vigour of different varieties of alfalfa.

Thus, the objectives of this research are to compare the changes in germination percentage, germination index, mean germination time and seedling vigour index in different varieties of alfalfa after boron priming, to evaluate the response of boron priming on seed vigour in different varieties of alfalfa and to accurately provide an exercisable, effective and economic method for the application of boron in alfalfa seeds' production.

MATERIALS AND METHODS

Seed source

Thirteen varieties of alfalfa seeds were used in this experiment, including Pianguan, WL168HQ, WL298HQ, WL319HQ, WL343HQ, WL354HQ, WL363HQ, WL366HQ, WL440HQ, WL525HQ, WL656HQ, WL712 and WL903. Alfalfa (variety: Pianguan) seeds were collected by the Forage Seed Laboratory of Shanxi Agricultural University in

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How to cite this article: Xia, F.S., Wang, Y.C., Wang, F., Wang, C.C., Zhu, H.S., Liu, M., Huai, Y.M., Dong, K.H. (2020). Effect of Boron Priming on the Seed Vigour in Different Varieties of Alfalfa (*Medicago sativa* L.). Legume Research. 43(3): 415-420.

Submitted: 08-03-2019 **Accepted:** 12-05-2020 **Published:** 23-06-2020

August 2017 and then sealed in plastic bags and stored at -20°C. Other varieties were provided by Beijing Rytway Ecotechnology Co., LTD. The experiment was launched on July 2018 in the Forage Seed Laboratory of Shanxi Agricultural University.

Boron priming treatments

Alfalfa seeds were soaked in 1.8% (W/V, selected according to 0, 0.1, 0.3, 0.6, 1.2, 2.4 and 4.8%) concentration of borax solution for 0, 3, 6, 12 and 24 h at 20°C. Thereafter, seeds were immediately scoured two times with deionized water and surface moisture were removed using filter paper. They were then air-dried for three days in the dark at 25°C and 45% RH in order to ensure moisture content reached approximately 10% (on fresh weight basis). Each treatment had four replicates.

Germination tests

Germination tests were executed following the ISTA rules (2017). Hundred seeds from each sample were selected and placed into petri dishes that was lined with three filter papers and wetted with 10 ml deionized water. They were then placed in constant temperature incubators at 20°C. Germination was recorded daily based on 2 mm radicle growth through seed coat. The number and length of normal seedlings were counted in each petri dish on day-10. Germination percentage was computed by the method of ISTA (2017). Germination index and seedling vigour index were calculated according to Abdul-Baki and Anderson (1973). Mean germination time was counted according to Ellis *et al*. (1982). The full formula had been reported in our previous studies (Xia *et al*., 2019).

Statistical analyses

Seed germination data was respectively tested using the Kolmogorov-Smirnov test and the homogeneity test of variances and was conformed to normal distribution and homoscedasticity. Mean difference of comparisons were performed using an analysis of variance (ANOVA), which was conducted using SPSS for Windows ver. 13.0 followed by Duncan's multiple range test (P = 0.05).

RESULTS AND DISCUSSION

Effects of boron priming on germination percentage

The initial germination percentage of alfalfa seeds was different across varieties and their variations were also different with the extension of boron priming times (Table 1). There were highly significant (P<0.01) differences among varieties, priming time and their interaction on germination percentage of alfalfa seeds (Table 2). In this study, germination percentage of WL525HQ and WL656HQ declined significantly (P<0.05) with increasing length of priming time, which manifested that priming with 1.8% boron caused a decrease in the germination percentage of alfalfa seeds in both varieties. Excessive boron could inhibit both

Table 1: Effect of boron priming on germination percentage in different varieties of alfalfa seeds.

Varieties	Time (h)				
	0	3	6	12	24
WL168HQ	86±0.82CDa	86±0.94Ba	73±0.99Gb	67±0.99Gc	53±1.04CDd
WL298HQ	83±0.47DEa	83±0.58CDa	81±0.82CDEa	75±0.67CDb	42±0.98Hc
WL319HQ	81±0.55EFa	82±0.87CDa	82±0.75CDa	77±0.55Cb	45±0.75GHc
WL343HQ	85±0.94CDa	85±0.75BCa	84±1.11BCa	75±1.06CDb	52±0.92Dc
WL354HQ	89±0.82Bab	87±0.67Bb	91±0.87Aa	86±0.96Ab	49±1.11EFc
WL363HQ	93±0.75Aa	91±0.55Aa	87±1.20Bb	81±0.82Bc	52±1.06Dd
WL366HQ	78±0.94Gb	82±0.82CDa	80±0.94DEab	70±1.00FGc	46±0.82FGd
WL440HQ	86±0.73CDa	83±0.55CDa	79±1.08DEb	74±0.99DEc	53±0.47CDd
WL525HQ	86±0.99CDa	80±0.96Db	78±0.94EFc	74±0.58DEd	68±0.99Ae
WL656HQ	92±0.58ABa	86±0.75Bb	79±0.67DEc	71±0.67EFd	62±0.96Be
WL712	74±1.06Ha	75±0.87Ea	69±0.87Hb	64±0.82Hc	51±0.87DEd
WL903	80±0.67FGa	82±0.94CDa	76±0.75FGb	72±0.55EFc	56±1.13Cd
Pianguan	78±0.75Gb	82±0.75Da	79±0.55DEab	70±0.75FGc	63±0.55Bd

Note: Means in the same column with different capital letters are significant difference (*P*<0.05), in the same row with different small letters are significant difference (*P*<0.05).

Table 2: Variance analysis of priming times and varieties on alfalfa seed vigour.

Source	Degree of freedom	Gp		MGT		Gi		SVI	
		F value	Sig.	F value	Sig.	F value	Sig.	F value	Sig.
Varieties	12	135.178	0.000	756.107	0.000	628.765	0.000	1439.718	0.000
Times	4	3938.891	0.000	40173.316	0.000	17865.301	0.000	23201.701	0.000
Varieties×Times	48	44.1385	0.000	346.257	0.000	163.536	0.000	323.570	0.000

Note: Gp: germination percentage; MGT: mean germination time; Gi: germination index; SVI: seedling vigour index.

the vegetative and reproductive growth of plants (Kaya and Ashraf, 2015). Also, our previous reports showed that the germination percentage of alfalfa seeds could be markedly reduced with immoderate boron priming (Xia *et al*., 2019). Therefore, these results showed that the seeds of WL525HQ and WL656HQ might be sensitive to boron toxicity in terms of germination percentage. Nonetheless, the germination percentage of WL366HQ and Pianguan reached the maximum level at 3 h, which was significantly (P<0.05) higher than those unprimed seeds, this proved that priming with 1.8% boron promoted the germination percentage of alfalfa seeds in both varieties. Li *et al*. (2017) found that seed priming could improve the germinating ability of alfalfa seeds. Similarly, previous studies also indicated that boron priming could effectually promote seed germination (Deb *et al*., 2010; Iqbal *et al*., 2017). Beyond this, a significant (P<0.05) decrease occurred in those seeds primed for 12 and 24 h. These results indicated that the seeds of WL366HQ and Pianguan might be insensitive to boron toxicity in terms of germination percentage. This was similar to our previous reports in those seeds primed with 1.2 and 2.4% boron (Xia *et al*., 2019). Likewise, strong boron priming could not promote the seed germination (Farooq *et al*., 2012). In this study, the difference in germination percentage was not significant (P>0.05) between those primed for 0 and 3 h in other varieties of alfalfa seeds. Particularly, the germination percentage of WL319HQ, WL343HQ and WL354HQ still maintained a high level after being primed at 0, 3 or 6 h and there were no significant (P>0.05) differences among these seeds. These results showed that these varieties were moderately sensitive to boron toxicity in terms of germination percentage.

Effects of boron priming on germination index

The original germination index of alfalfa seeds was disparate in different varieties and their changes were also varies with the extension of boron priming times (Table 3). There were highly significant (P<0.01) differences among varieties, priming time and their interaction on germination index of alfalfa seeds (Table 2). Seed priming had been regarded as an effective method to improve germination index of seeds (Xia *et al*., 2017). In this study, the germination index of WL903 and Pianguan reached the maximum level at 3 h and they were significantly (P<0.05) higher than others. These results indicated that the germination index of alfalfa seeds in both varieties were promoted by priming with 1.8% boron. This might be attributed to the complement of antioxidant systems and ultrastructure of embryonic cells during seed priming (Xia *et al*., 2017). Moreover, a significant (P<0.05) decrease occurred in these two varieties of alfalfa seeds primed for 6 to 24 h. Therefore, these results showed that these two varieties of alfalfa seeds were insensitive to boron toxicity as far as germination index. Nonetheless, the germination index reduced markedly (P<0.05) with the extension of priming time in other varieties of alfalfa seeds, which showed that these seeds might be more sensitive to boron toxicity according to their germination index.

Effects of boron priming on mean germination time

Seed priming has been deemed to activate hydrolytic enzymes and improve embryonic physiology, thereby their germination happen in less time (Bam *et al*., 2006). Additionally, boron could promote the remobilization of their stored nutrients during seed germination (Bonilla *et al*., 2004). In this study, there were highly significant (P<0.01) differences among varieties, priming time and their interaction on mean germination time of alfalfa seeds (Table 2). The mean germination time of WL903 reached the minimum level at 3 h, which was significantly (P<0.05) lower than others (Table 4). These results showed that the germination speed of WL903 was accelerated by priming 3 h with 1.8% boron. Nevertheless, they decreased

Table 3: Effect of boron priming on germination index in different varieties of alfalfa seeds.

Varieties	Time (h)				
	0	3	6	12	24
WL168HQ	25.56±0.23Ga	23.40±0.21BCb	17.90±0.15Fc	15.67±0.23Fd	11.58±0.02Ee
WL298HQ	26.90±0.17Fa	23.13±0.22BCDb	18.67±0.42EFc	17.80±0.12Cc	8.18±0.13Ed
WL319HQ	32.25±.014Ca	20.38±0.20Gb	18.85±0.33DEc	16.05±0.16EFd	9.00±0.09Ge
WL343HQ	28.08±0.10Ea	22.19±0.21Eb	19.75±0.24CDc	16.97±0.09Dd	9.43±0.11Ge
WL354HQ	30.01±0.19Da	23.83±0.24Bb	20.77±0.26ABc	17.61±0.20Cd	10.34±0.09Fe
WL363HQ	32.06±0.13Ca	22.75±0.22CDEb	19.73±0.26CDc	17.78±0.23Cd	10.59±0.25Fe
WL366HQ	23.42±0.20Ia	21.23±0.17Fb	18.90±0.287DEc	16.24±0.06Ed	10.07±0.12Fe
WL440HQ	34.50±0.12Ba	27.15±0.32Ab	21.50±0.12Ac	18.68±0.10Bd	13.54±0.17De
WL525HQ	30.11±0.13Da	23.83±0.13Bb	19.14±0.43CDEc	18.96±0.15Bc	15.43±0.18Bd
WL656HQ	35.15±0.16Aa	27.21±0.05Ab	19.41±0.39CDEc	19.81±0.11Ac	14.51±0.16Cd
WL712	24.14±0.12Ha	22.54±0.24DEb	16.03±0.10Gc	14.09±0.18Gd	11.40±0.19Ee
WL903	20.85±0.20Jb	22.67±0.07DEa	16.14±0.09Gc	14.29±0.13Gd	8.41±0.10He
Pianguan	21.35±0.17Jb	23.44±0.17BCa	20.03±0.11BCc	18.88±0.06Bd	16.06±0.17Ae

Note: Means in the same column with different capital letters are significant difference (*P*<0.05), in the same row with different small letters are significant difference (*P*<0.05).

significantly (P<0.05) after priming from 6 to 24 h. Moreover, mean germination time declined prominently (P<0.05) with the extension of priming time in other varieties of alfalfa. Seed priming was based on the relationship between imbibition and water potential (Bewley *et al*., 2013). However, the increase in concentration of priming solution usually leads to a decline in water potential delaying cell activation (Xia *et al*., 2017). Thus, these results illustrated that these varieties of alfalfa seeds might be more sensitive to boron toxicity in terms of mean germination time. This was similar to our previous reports (Xia *et al*., 2019). Farooq *et al*. (2011) also found that boron priming could distinctly delay the emergence of rice seeds.

Effects of boron priming on seedling vigour index

The level of seedling vigour index generally demonstrated the ability of seedling establishment in growth process. Iqbal *et al*. (2017) found that boron priming at low concentration resulted in seedlings with longer shoots and consequently able to acquire more nutrients. However, there were highly significant (P<0.01) differences among varieties, priming time and their interaction on seedling vigour index of alfalfa seeds (Table 2). In this study, seedling vigour index increased in the varieties of WL168HQ, WL298HQ, WL319HQ,WL343HQ, WL363HQ, WL366HQ, WL712 and Pianguan after 3 h of priming, which was significantly (P<0.05) higher than those of non-primed (Table 5). These results showed that seedling vigour index of these varieties were accelerated by priming 3 h with 1.8% boron. Particularly, seedling vigour index of WL298HQ, WL343HQ and WL354HQ remained at the highest level after 3 h of

Table 4: Effect of boron priming on mean germination time indifferent varieties of alfalfa seeds (days).

Varieties	Time (h)				
	0	3	6	12	24
WL168HQ	1.91±0.028Be	2.01±0.023BCd	2.18±0.007FGc	3.11±0.013Bb	3.82±0.022Ga
WL298HQ	1.80±0.026CDe	1.90±0.021DEd	2.34±0.012BCc	3.07±0.018Bb	5.20±0.012Aa
WL319HQ	1.42±0.022He	2.10±0.020Ad	2.32±0.018BCc	2.96±0.016Cb	4.55±0.023Da
WL343HQ	1.71±0.027DEFe	2.11±0.014Ad	2.21±0.016EFc	3.10±0.011Bb	4.43±0.015Ea
WL354HQ	1.71±0.025DEFe	1.94±0.023CDd	2.37±0.020Bc	2.93±0.014Cb	4.54±0.006Da
WL363HQ	1.68±0.022EFe	2.04±0.018ABd	2.31±0.012Cc	2.72±0.009Eb	4.64±0.024Ca
WL366HQ	1.86±0.018BCe	2.09±0.023Ad	2.26±0.015DEc	2.84±0.018Db	4.01±0.020Fa
WL440HQ	1.41±0.027He	1.87±0.019Ed	2.22±0.013EFc	2.44±0.015Gb	3.58±0.022Ha
WL525HQ	1.67±0.032EFe	1.94±0.028CDd	2.35±0.019BCc	2.45±0.027Gb	3.16±0.024Ja
WL656HQ	1.55±0.020Ge	1.79±0.025Fd	2.43±0.021Ac	2.87±0.016Db	3.96±0.009Fa
WL712	1.76±0.023DEe	1.98±0.015BCd	2.47±0.018Ac	3.09±0.026Bb	3.26±0.027Ia
WL903	2.15±0.027Ad	1.99±0.007BCe	2.31±0.016CDc	3.38±0.005Ab	4.87±0.020Ba
Pianguan	1.64±0.024Fe	1.89±0.011DEd	2.14±0.010Gc	2.57±0.013Fb	2.98±0.013Ka

Note: Means in the same column with different capital letters are significant difference (*P*<0.05), in the same row. with different small letters are significant difference (*P*<0.05).

Table 5: Effect of boron priming on seedlings vigour index in different varieties of alfalfa seeds.

Varieties	Time (h)				
	0	3	6	12	24
WL168HQ	42.92±0.21Gb	45.22±0.45Ga	35.93±0.43Hc	31.15±0.28Id	19.85±0.24Ee
WL298HQ	42.74±0.11GHb	46.22±0.59FGa	45.43±0.39Da	36.32±0.15Fc	14.67±0.20Hd
WL319HQ	39.28±0.25Jb	44.45±0.28Ga	38.55±0.51Gbc	37.46±0.27Ec	14.45±0.17Hd
WL343HQ	41.80±0.33Hb	45.75±0.37FGa	45.61±0.30Da	37.12±0.19Ec	18.16±0.13Fd
WL354HQ	49.64±0.13Eb	50.38±0.54Cb	57.20±0.26Aa	43.23±0.11Ac	18.46±0.15Fd
WL363HQ	46.98±0.17Fc	58.67±0.63Aa	48.73±0.09Bb	41.60±0.22Bd	21.12±0.12De
WL366HQ	37.27±0.25Kd	48.49±0.65DEa	43.51±0.14Eb	39.24±0.28Dc	16.47±0.21Ge
WL440HQ	56.84±0.13Ca	49.86±0.32CDb	43.04±0.24Ec	40.52±0.38Cd	20.39±0.24Ee
WL525HQ	64.81±0.37Aa	49.48±0.69CDb	47.54±0.15Cc	40.23±0.12Cd	38.82±0.25Ae
WL656HQ	61.22±0.49Ba	50.37±0.49Cb	45.65±0.19Dc	42.93±0.14Ad	36.13±0.21Be
WL712	40.74±0.57Ib	47.08±0.47EFa	40.28±0.10Fb	34.50±0.24Gc	18.68±0.14Fd
WL903	52.94±0.09Da	48.77±0.42CDEb	39.90±0.35Fc	33.56±0.15Hd	18.19±0.27Fe
Pianguan	50.53±0.18Eb	52.50±0.06Ba	40.28±0.21Fc	35.71±0.16Fd	23.46±0.22Ce

Note: Means in the same column with different capital letters are significant difference (*P*<0.05), in the same row with different small letters are significant difference (*P*<0.05).

priming, showing that these three varieties of alfalfa seeds were insensitive to boron toxicity. The successful establishment of a seedling relied largely on effective mobilization of storage nutrient (Sew *et al*., 2016). Hence, primed seeds might possess greater energy to complete seedling growth (Chen and Arora, 2013). Nevertheless, they all declined with the extension of time, which indicated that excessive boron priming might also inhibit the growth of alfalfa seedlings. Our previous study had similar results (Xia *et al*., 2019). Unlike these varieties, seedling vigour index of WL440HQ, WL525HQ, WL656HQ and WL903 declined with increased priming time, which illustrated that seedling vigour index in these varieties of alfalfa seeds might be more sensitive to boron toxicity.

CONCLUSION

There were highly significant ($P<0.01$) differences among varieties, priming time and their internation on germination percentage, germination index, mean germination time and seedling vigour index of alfalfa seeds. Additionally, the seeds of the varieties *viz*., WL525HQ and WL656HQ were more sensitive to boron toxicity, but the varieties *viz*., WL298HQ, WL343HQ, WL354HQ, WL903 and Pianguan were insensitive.

ACKNOWLEDGEMENT

This research was financially supported by the Natural Science Foundation of China (NSFC31702171), the key research and development program of Shanxi (201903D221091) and Science and Technology Innovation Fund of Shanxi Agricultural University (2016YJ15).

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