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Effects of Different Boron Doses on Germination, Seedling Growth and Relative Water Content of Linseed (*Linum usitatissimum* L.)

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HIGHLIGHTS

- In the study was to determine the effects of boron doses on linseed cultivars.
- Many parameters have been studied and the results are important.
- Boron applications decreased germination, seedling growth and relative water content.
- It was determined that 8 ml L^{-1} of the applied boron doses had a toxic effect.

Abstract

Boron is one of the most important nutrients required for the growth and development of plants. However, boron deficiency or excess also affects the physiological development of plants such as germination and seedling development. In this study, the effects of boron applications of 0-8 ml L^{-1} (4 concentrations) in 2 linseed cultivars [Beyaz Gelin (C1) and Sarı Dane (C2)] were investigated under laboratory conditions. In the study; germination percentage (GP), mean germination time (MGT), seeding length (SL), root length (RL), seedling fresh weight (SFW), seedling dry weight (SDW), root fresh weight (RFW), root dry weight (RDW) and relative water content (RWC) parameters were examined. As a result of the study, it has been determined that there are decreases in the properties of the cultivars in terms of the parameters examined with boron applications. It has been determined that especially 8 mg L^{-1} application has a toxic effect and prevents seedling and root development.

Keywords: boron, linseed, *linum usitatissimum* L., plant tolerance mechanism, RWC

1. Introduction

Plant nutrients are the elements that are absolutely necessary for the development of plants (Gezgin and Hamurcu 2006). Nutrient elements are divided into two as macro and micro elements according to the needs of plants, and macro nutrients are elements that are needed more than micro elements. Micronutrients are also called trace elements because plants need small amounts. Macro nutrient elements are carbon, hydrogen, oxygen, nitrogen, potassium, calcium, phosphorus, magnesium and sulfur, and micronutrients are iron, chlorine, copper, manganese, zinc, molybdenum, boron and nickel (Bolat and Kara 2017).

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Boron is a micro nutrient element that is absolutely essential for plants (Welch and Shuman 1995; Prathima et al. 2016; Beyaz et al. 2018). In addition, boron is one of the nutrients required in different amounts for the development of all plants (Demirtaş 2005). While different concentrations of boron are excessive for some plants, they may be below the desired amount for some plants (Yolci et al. 2022). Due to these different concentrations, boron deficiency or being in a toxic state occurs in plants and affects the activities of antioxidant enzymes in various ways. However, too much or too little boron element causes oxidative stress in plants (Hamurcu et al. 2015). In plants, boron deficiency causes decreased product quality and losses, while boron excess causes toxicity (Tassi et al. 2017). In addition, excess boron slows down growth and development in plants, causes deterioration of leaf morphology and transpiration, reduction of cell division in roots and ultimately oxidative stress (Karabal et al. 2003; Kacar and Katkat 2006). It has been reported by researchers that a sufficient level of boron element plays an important role in increasing the resistance against abiotic stresses under stress conditions.

Boron element in plants is involved in the cell membrane and wall, the activity of many enzymes, and the transport of metabolites, hormones and various ions produced as a result of biochemical processes (Dordas et al. 2000). At the same time, boron is a nutrient element that has a wide range of effects on the physiological development processes of plants (Ayvaz et al. 2016). Boron is used in agriculture to increase the development of vegetation (Demirtaş 2006). Seed treatment with boron helps in improving seed germination percentage, shoot and root length, early seedling vigor etc. helps in better early crop growth (Goldberg and Glaubig 1985). Besides this seed germination and seedling development are very important and critical stages in terms of crop production (Almansouri et al. 2001).

Linseed is a plant with varieties that can be grown for its fiber and oil (Hazneci and Arslanoğlu 2021). It is one of the oldest plants grown all over the world for flax fiber (Arslanoglu et al. 2022). In addition, linseed grown for oil production is rich in alpha-linoleic acid and rich in omega-3 fatty acids (Eseceli et al. 2006; Gogus and Smith 2010; Baydar and Erbaş 2014; Gürsoy 2019; 2022).

The aim of this study was to determine the effects of boron doses on germination, seedling growth and relative water content in linseed cultivars.

2. Materials and Methods

This research was carried out in Namık Kemal University Field Crops Department Laboratories. The experiment was arranged in completely randomized design with three replications. Seeds of linseed for surface sterilization, they were kept in 5% sodium hypochlorite solution for 10 minutes and then rinsed several times in distilled water then they were dried at room temperature to their initial weight. In the study, 2 linseed cultivars [Beyaz Gelin (C1) and Sarı Dane (C2)] obtained from the Trakya Agricultural Research Institute were used, and 4 doses of boron (0, 2, 4 and 8 mg L^{-1}) were applied and deionized water was used for the control treatment. Oil content and 1000 seed weight of the linseed cultivars are between 27.8-30.6% and 5.7-6.6 g, respectively. In addition, the seed color of Beyaz Gelin cultivar are brown, the seed color of Sarı Dane cultivar is yellow. Boron is obtained from water-soluble 8% w/w Boron Ethanolamine (Boron-8, Gubretas). For each boron dose, 20 seeds were placed in sterile petri dishes on Whatman No: 1 blotting papers and 5 mL of different doses of boron concentrations were added on March 14, 2023. Only deionized (DI) water was added to the control petri dish. Filter papers were changed every 2 days and 5 mL of boron containing solutions were added. Petri dishes are wrapped with parafilm and kept at room temperature (24 ± 2 °C). Seeds were counted daily and those with a root length of 2 mm were considered germinated (ISTA 2003). In the study; germination percentage (GP), mean germination time (MGT), seedling length (SL), root length (RL), seedling fresh weight (SFW), seedling dry weight (SDW), root fresh weight (RFW), root dry weight (RDW) and relative water content (RWC) parameters were examined.

Measurements

Germination Percentage (%)

Given formula was applied to calculate germination percentage.

Germination % = (number of germinated seeds / total number of seeds) × 100 (Siddiqi et al. 2007)

Mean Germination Time (day)

MGT= $\Sigma(Dn)/\Sigma n$

where, n is the seed number germinated on day (D), and D is the number of days from the beginning of the germination test (Orchard 1977).

Seedling Length (cm)

The seedlings and roots of the plants in each petri dish were separated from each other 10 days after germination and determined by weighing the seedling part on a precision scale.

Root Length (cm)

It was determined by weighing the roots of the plants whose seedling and root parts were separated.

Seedling and Root Fresh Weight (g)

It was determined by weighing the seedlings and roots of control and boron-treated plants on sensitive scales.

Seedling and Root Dry Weight (g)

Seedling and root dry weights were recorded after oven drying for 48 h at 55 ^{o}C (Ateş and Tekeli 2007).

Relative Water Content (%)

Leaf samples taken from the plants in the control and stress groups were weighed and their fresh weights were determined, and placed in glass tubes containing 5 mL of deionized water and left in the light for 24 hours. At the end of this period, the hydrated leaf samples were weighed again and their turgor weights were determined, and then these leaf samples were dried in an oven at 80 ^{o}C for 48 hours and their dry weights were determined again. Finally, the relative water contents were calculated according to the following formula (Ritchie et al. 1990).

RWC (%) = (FW − DW) / (TW − DW) × 100

FW: fresh weight, TW: turgor weight, DW: dry weight

Results was analyzed using TARIST and MSTAT-C (MSTAT 1989) statistical software for analysis of variance. Least Significant Difference (LSD) test was used to compare the means of the obtained results in this research (p<0.05).

3. Results and Discussion

The variance analysis results of this study, which was conducted to determine the effects of boron doses on the germination parameters, seedling and root growth, relative water content of linseed cultivars is given in Table 1. When Table 1 is examined, it is seen that the doses in the GP, MGT, RFW and RWC parameters, the doses and cultivars in the SL, RL, SFW, SDW parameters, and the cultivars in the RDW feature made a statistically significant difference by 5%. SL, RL, SFW, SDW, RFW, RDW and RWC parameters were not included in the statistical calculation and 0 was accepted as there was not sufficient germination and seedling growth in both cultivars at the 4th dose of boron applications.

Table 1. Average values the effect of boron at different concentrations applied to linseed cultivars

Parameters	Cultivars	Doses				Means
		0 mg L^{-1}	2mg L^{-1}	4 mg L^{-1}	8 mg L^{-1}	
GP (%)	C1	98,33	68,33	28,33	8,33	50,83
	C2	96,67	61,67	16,67	8,33	45,83
	Means	97,50 **a**	65,00 **b**	22,50 **c**	8,33 **d**	
	$LSD_{0,05}$:	Dose: 4,973				
MGT (day)	C1	2,63	4,83	6,73	8,00	5,55
	C2	2,77	5,27	7,00	7,33	5,59
	Means	2,70 **d**	5,05 **c**	6,87 **b**	7,67 **a**	
	$LSD_{0,05}$:	Dose: 0,623				
SL (cm)	C1	5,13	5,03	3,20	0	4,45 **a**
	C2	4,27	4,03	2,60	0	3,63 **b**
	Means	4,70 **a**	4,53 **a**	2,90 **b**	0 **c**	
	$LSD_{0,05}$:	Cultivar: 0,619	Dose: 0,443			
RL (cm)	C1	8,40	7,20	5,03	0	6,88 **a**
	C2	5,20	5,30	2,93	0	4,48 **b**
	Means	6,80 **a**	6,25 **a**	3,98 **b**	0 **c**	
	$LSD_{0,05}$:	Cultivar: 0,562	Dose: 1,008			
SFW (g)	C1	0,047	0,042	0,038	0	0,042 **a**
	C2	0,033	0,027	0,026	0	0,029 **b**
	Means	0,040 **a**	0,034 **b**	0,032 **c**	0 **d**	
	$LSD_{0,05}$:	Cultivar: 0,003	Dose: 0,002			
SDW (g)	C1	0,022	0,019	0,016	0	0,019 **a**
	C2	0,016	0,012	0,011	0	0,013 **b**
	Means	0,019 **a**	0,016 **b**	0,013 **b**	0 **c**	
	$LSD_{0,05}$:	Cultivar: 0,002	Dose: 0,003			
RFW (g)	C1	0,018	0,014	0,010	0	0,014
	C2	0,013	0,012	0,012	0	0,012
	Means	0,015 **a**	0,013 **ab**	0,011 **b**	0 **c**	
	$LSD_{0,05}$:	Cultivar: 0,002				
RDW (g)	C1	0,007	0,006	0,006	0	0,006 **a**
	C2	0,003	0,004	0,002	0	0,003 **b**
	Means	0,005	0,005	0,004	0	
	$LSD_{0,05}$:	Cultivar: 0,001				
RWC (%)	C1	68,50	63,97	60,13	0	64,20
	C2	67,67	63,67	60,67	0	64,00
	Means	68,08 **a**	63,82 **b**	60,40 **c**	0 **d**	
	$LSD_{0,05}$:	Dose: 1,230				

*: Dissimilar letters in the column show different group

When Table 1 is examined, it was determined that the most germination in the GP feature was determined in the control application, and it was determined that the germination decreased as the application doses increased. It is observed that this decrease occurred at the rate of 90 and 88% in C1 and C2 cultivars, respectively. However, it was determined that the highest decrease was in the 3rd dose. Taban and Erdal (2000) reported that durum wheat cultivars were more affected by boron than bread cultivars in wheat cultivars to which boron was applied. Ashagre et al. (2014) reported that in safflower a significant decrease was observed on germination percentage as boron concentrations increased beyond 2 mg l^{-1} concentration. Gökkaya and Arslan (2023) reported that low dose boron applications caused an increase in germination and seedling growth parameters in their study where they applied boron to the seeds of sorghum plant under drought stress conditions.

In Table 1, which includes the averages of the MGT parameter, is examined, the highest average germination time was determined 4. dose. The lowest MGT was obtained from control application. It was determined that MGT decreased of boron applications, however, it was observed that MGT increased with increasing boron doses. Culpan et al. (2019) reported that as boron doses increased, the number of germination days increased as well, in their study where they applied boron doses to safflower cultivars. Gökkaya and Arslan (2023), in their study in which they applied boron to sorghum seeds under drought conditions, reported that MGT (3.66, 3.62, 3.67, 3.85 days) with increasing boron doses (0, 5, 10, 15 mM) increased in general even though it decreased slightly at the 5 mM dose.

When Table 1 was examined in terms of SL, it was determined that the seedling length was shortened with increasing boron doses, and the seedling height could not be measured at the 4th dose. However, after the second dose, the shortening of the seedling appeared more clearly. Culpan et al. (2019) used doses of (0, 0.5, 1, 1.5 mg L^{-1}) in their study where they applied boron doses to safflower cultivars. They reported that the seedling length was determined as (4.13, 4.25, 3.49, 4.80 cm), respectively. The results of the researchers differ from the results obtained in this study. The reason for this is the diversity of species, lower doses applied, etc. considered to be due to reasons such as Donbaloğlu Bozca and Leblebici (2022) applied boron to sunflower plant under heat stress conditions. As a result of the study, boric acid and heat stress increased the negative effect seedling length, while, a positive effect of boric acid application at low temperature on the seedling length of the plant. They reported that boron application at low temperature caused the seedling length to grow.

When Table 1 was examined in terms of RL, it was determined that RL decreased as the boron doses increased in the C1 cultivar, and in C2, although it increased a little in the 2nd dose compared to the control, it decreased again in the 3rd dose. In the 4th dose of boron application, there was no root development and root length could not be determined. With these results, it was determined that the root length shortened as the doses of boron applications increased. C2 cultivar was more affected by boron applications and root length was shorter than C1 cultivar. Akçam-Oluk and Demiray (2004) reported that root length increased in excess of boron in their study in which they applied boron in sunflower cultivar. Eroğlu and Topal (2022) reported that increasing concentrations of solutions negatively affect the stem and root length values and fresh-dry weight of plants as a result of a study in which they grew barley using solutions prepared from wastes with high boron content, and this negative effect is more pronounced when waste with higher boron content is used.

As seen in Table 1, SFW decreased as boron doses increased in both cultivars. However, it was determined that this effect was higher in C2 cultivar than in C1. However, it is seen that there is no plant growth and SFW cannot be determined at the 4th dose of boron applications. It is thought that the decrease in SFW is due to the fact that B doses reach toxic levels and accordingly plant growth is negatively affected. Similar reduction in shoot fresh weight with the increasing level of B was also observed in tomato and pepper (Eraslan et al. 2007). Seferoğlu and Kaptan (2020) reported that the fresh and dry weights and % dry matter values of barley and wheat plants grown with irrigation water containing different levels of boron decreased compared to the control as the boron concentration increased, and they determined the lowest at the highest dose (5 mg L^{-1}).

In terms of SDW (Table 1), there is a situation similar to SFW. It was determined that SDW decreased as boron doses increased, it could not be determined at all in the 4th application dose, and C2 cultivar was affected more than C1 by this decrease. This situation emerged as a decrease in SDW in parallel with the decrease in SFW with the increase of boron doses. Arslan et al. (2022) investigated the effects of salt pre-treatments on boron toxicity in safflower cultivars. The fresh and dry weight of shoot (14-53% and 34-61%, respectively) decreased at 4 mM and higher B treatments. It is thought that this decrease is due to the toxic effect that occurs with the increase in boron applications.

When Table 1 is examined in terms of RFW, it is seen that RFW decreases as the boron doses increase compared to the control, and it cannot be determined at all at the highest boron dose of 8 mg L^{-1}. Therefore, root development did not occur in the plant after a certain dose of boron. Muhammad et al. (2013) reported that root fresh and dry weight of maize plant decreased with increasing boron doses. Similarly, Turan et al. (2009) reported that root fresh and dry weights wheat decreased with the increase in concentrations of boron. Culpan et al. (2019) applied boron doses to safflower cultivars and similarly reported that RFW decreased as boron doses increased. In the study of Donbaloğlu Bozca and Leblebici (2022) in which they applied boron to sunflower plant under temperature stress conditions, it was determined that high temperature significantly reduced root biomass.

In terms of RDW (Table 1), although there was a decrease in C1 cultivar compared to the control, boron doses were determined to be the same in the 2nd and 3rd application doses (2 and 4 mg L^{-1}), and RDW could not be determined in 8 mg L^{-1} application in both cultivars. In the C2 cultivar, although there was a slight increase in the 2 mg L^{-1} dose compared to the control, it was determined that RDW decreased again at the 4 mg L^{-1} dose. A negative impact of high B was explained as the result of prevented plant growth, which might be because of the toxic effects on root cell division (Çelik et al. 2019). Culpan et al. (2019) and Arslan et al. (2022) reported that RDW decreased with increasing boron doses in their study on safflower.

When Table 1 was examined in terms of RWC, it was determined that the relative water content of plants decreased with increasing boron doses compared to the control. However, similar results were obtained in both cultivars. It is seen that the cultivars are affected by boron doses at almost the same level in terms of RWC. Arslan et al. (2022) reported that RWC decreased with the applied boron doses in their study where they performed salt pre-application in safflower cultivars.

4. Conclusions

In this study, the effects of different doses of boron applications on linseed cultivars seeds were determined. As a result of the study, it was determined that boron applications had negative effects on the germination parameters, early seedling growth, relative water content of linseed. It was determined that with the increase of boron dose, seedling and root development did not occur in both cultivars. In terms of other parameters examined, it was determined that the properties of the cultivars decreased with increasing boron doses. Besides, applications should be made in other plants and their results should be evaluated.

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