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RESEARCH ARTICLE

Effects of Drought Stress on Germination and Seedling Growth of Seed Primed with Boron in Spinach

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ABSTRACT

This study aimed to examine the effects of seed priming with different boron concentrations on the germination and seedling growth of spinach under drought-stress conditions. Seeds of the spinach cultivar Matador and sodium borate ($Na_2B_8O_{13}.4H_2O$) were used as materials. The seeds were primed with 0 (distilled water), 1, 10, 100, 500, and 1000 ppm B for 24 hours, with unprimed seeds serving as a control. Drought stress was induced by polyethylene glycol (PEG 6000) solution at a water potential of -3 bar and distilled water denoted as control conditions. A standard germination test was performed between papers at 20°C for 14 days. The germination percentage, mean germination time, germination index, seedling growth parameters, and root/shoot length ratio were investigated. The findings revealed that drought stress reduced germination percentage, germination index, and seedling growth of unprimed seeds of spinach. However, boron priming improved these parameters while mitigating the negative effects of drought stress. Under drought conditions, seed priming with 1 ppm B shortened mean germination time. Similarly, seedling fresh and dry weight of spinach were improved by seed priming with 100-1000 ppm B, whereas root growth was stimulated by 10 ppm B. The highest root/shoot ratio was found at 10 ppm B. Boron priming was more efficient in promoting seedling growth than germination in spinach. As a result, seed priming with 10-100 ppm B should be recommended to improve the germination and seedling growth performance of spinach in the event of drought stress after planting.

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1. Introduction

Spinach (*Spinacia oleracea* L.) is a cool-season annual crop grown for its leaves, which are consumed fresh and frozen. Its leaves are rich in minerals, antioxidants, and vitamins such as A, B, and C (Bunea et al., 2008). In Türkiye, its production is about 232.699 tons, with an area of 15.447 ha in 2023 (TÜİK, 2024). Spinach is grown from late summer to early winter in temperate regions and from late winter to spring in cold climates. Under these conditions, drought stress occurs during the life cycle of the spinach plant and has adverse effects on germination, emergence, and plant growth, such as short and small leaves that turn green to yellow (Vural et al., 2000).

Drought inhibits and delays seed germination by preventing water uptake and radicle emergence. Inadequate soil moisture can greatly hinder successful seed germination and emergence (Saha et al., 2022). Moreover, seeds germinating under drought stress often resulted in reduced vigor, and a lower germination index (Tang et al., 2019). To mitigate the adverse effects of drought stress, seed priming is a promising technique that enhances early mobilization of seed reserves, embryo elongation, endosperm weakening, etc. to increase and accelerate seed germination under various abiotic stresses (Chen & Arora, 2011; Kumar et al., 2020).

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Seed priming is a pre-sowing process in which seeds are soaked in water or various solutions containing different natural or synthetic priming agents (gibberellic acids, IBA, BA, glycine betaine, etc.) and dried again for storage (Bradford, 1986; Jisha et al., 2013; McDonald, 2000; Pallaoro et al., 2016; Waqas et al., 2019). In nutrient seed priming, seeds are soaked in a nutrient solution instead of pure water to improve the nutrient content of the seed in combination with the priming effect, which improves germination and seedling establishment (Imran et al., 2013, 2021; Iqbal et al., 2017). Priming solutions containing nutrients, such as nitrogen, calcium, manganese, zinc, and boron, called nutripriming, have been successful and responsive in vegetables (Kaur et al., 2002). In recent studies, it was discovered that nutripriming with different micronutrients enhanced priming efficiency in wheat (Iqbal et al., 2017), maize (Muhammad et al., 2015; Nciizah et al., 2020; Rasool et al., 2019), dill (Mirshekari, 2012), broccoli (Memon et al., 2013), rice (Farooq et al., 2011), bean (Majda et al., 2019), and rice (Ancy et al., 2022).

Boron (B), one of the most widely used micronutrients for seed priming, is important for cell division elongation, translocation, and membrane integrity (Iqbal et al., 2012). It has been extensively studied to improve the germination potential in several crops. For example, Farooq et al. (2011) reported that seed priming with B enhanced germination energy, percentage, and index, while shortening mean germination time in rice seeds. Bonilla et al. (2004) demonstrated that the application of B priming improved the germination characteristics and salt tolerance of developing pea plants. Kaya and Ergin (2023) reported that a lower infection rate was observed in safflower seeds primed with boron. Moreover, it was reported that the seedlings produced from B primed seeds grew better and were more resistant to heat stress due to lower ROS production, along with strong membrane stability and antioxidant defense system (Chakraborty & Dwivedi, 2023). Increases in antioxidant activities such as CAT, SOD, and hydrogen peroxidases were also observed in B-primed alfalfa seeds (Xia et al., 2020). Chakraborty and Bose (2020) found that seed priming with boron resulted in uniform and vigorous seedling establishment because they had much higher α-amylase activity compared to unprimed seeds. However, overdose and prolonged priming time with B resulted in a reduction in germination and a depression in seedling growth (Shahverdi et al., 2017; Xia et al., 2019). In this study, the efficiency of different levels of boron as a priming agent on germination and seedling growth of spinach under drought stress was investigated.

2. Materials and Methods

A laboratory experiment was conducted at the Seed Science and Technology Laboratory of Eskişehir Osmangazi University in 2023. Commercially available spinach (*Spinacia oleracea* L.) cultivar Matador seeds from the Arzuman Seed Company, Türkiye, and sodium borate (20.9% $Na_2B_8O_{13}\ 4H_2O$, Etidot-67) were used as materials.

2.1. Seed Treatments

The spinach seeds were immersed in the solutions with different boron concentrations (1, 10, 100, 500, and 1000 ppm B) using sodium borate in an incubator at 20 °C for 24 hours in the dark. Seeds were also soaked in distilled water (hydration), and unprimed seeds were used as a control. Following the incubation period, excess water on the seed surface was directly removed with paper towels, and they were led to dry up to their initial seed weight at room temperature.

2.2. Germination Test

ISTA (2018) rules were followed for the germination test, with four replications and fifty seeds in each. The unprimed and primed seeds were spread on two layers of filter paper, with one paper covering them. Each paper was moistened either with 7 mL of a solution prepared with polyethylene glycol (PEG 6000 m.w.) at a water potential of -3 bar as drought stress or with distilled water as control conditions (Michel & Kaufmann, 1973). To minimize water evaporation, the rolled papers were placed inside a plastic ziplock bag, and then the packages were incubated at 20 °C in the dark and checked every 24 hours. Seeds with a radicle length of 2 mm were considered to have germinated. At the end of the experiment (14th day), mean germination time (MGT), as determined by ISTA (2018), was computed to determine germination speed as follows.

$$MGT = \frac{\Sigma(\mathrm{Dn})}{\Sigma \mathrm{n}} \quad (1)$$

where n is the number of seeds that germinated on day D, and D is the number of days since the germination test started.

Also, the germination index (GI) was calculated with the formula (Salehzade et al., 2009).

$$\mathrm{GI} = \frac{\text{Number of germinated seeds}}{\text{Days of first count}} + \ldots + \frac{\text{Number of germinated seeds}}{\text{Days of final count}} \quad (2)$$

Root and shoot length, seedling fresh and dry weight of seedlings after 14 days of incubation were measured from ten seedlings randomly selected from each treatment. Just after weighing the fresh seedlings, they were transferred to an oven at 80 °C for 24 h to determine the dry weight.

2.3. Statistical Analysis

The experiment was set up as a factorial experiment in randomized plots design with 4 replications, and all data collected were statistically analyzed using the JMP 14.0 software. The percentage data were subjected to an arcsine transformation before an analysis of variance was performed. The LSD test was used to assess the differences between the means (Düzgüneş et al., 1983).

3. Results and Discussion

Mean values of germination characteristics showed that drought had no effect on germination percentage, however, it did result in longer mean germination time and a lower germination index (Table 1). Boron levels also significantly influenced the germination characteristics. Seed priming with a boron level of 1 ppm resulted in the highest germination percentage and the shortest mean germination time. Germination index reached the highest level with 17.33 in hydration and 17.20 in 1 ppm B.

Table 1. Main effects of seed priming with different levels of boron and drought stress on germination parameters of spinach.

Factors	Germination percentage (%)	Mean germination time (day)	Germination index
Stress			
Control	93.9	3.02^{b}	18.76^{a}†
Drought	92.3	4.00^{a}	13.09^{b}
B priming			
Unprimed	90.0^{d}	4.75^{a}	10.91^{f}
Hydration	95.3^{ab}	3.12^{c}	17.33^{a}
1 ppm B	95.8^{a}	3.10^{c}	17.20^{ab}
10 ppm B	92.5^{bcd}	3.23^{c}	16.44^{bc}
100 ppm B	92.0^{cd}	3.34^{c}	16.11^{cd}
500 ppm B	91.5^{d}	3.31^{c}	15.50^{d}
1000 ppm B	94.8^{abc}	3.75^{b}	14.50^{e}
Analysis of Variance			
Stress (A)	NS	**	**
Treatment (B)	**	**	**
A×B	**	**	NS

†: Means followed by the same letter(s) in each column did not significant at $p<0.05$. **: significant at 1%. NS: not significant.

All the seedling growth parameters were significantly influenced by the interaction of drought x boron priming (Table 2). As expected, lower seedling parameters were obtained from drought stress than the control, with the exception of seedling dry weight. Seed priming with different boron levels enhanced the seedling growth of spinach. Hydrated seeds produced the longest root length, while the highest seedling fresh at 1000 ppm B and dry weight at 100 ppm B were determined.

Table 2. Main effects of seed priming with different levels of boron and drought stress on seedling growth parameters of spinach.

Factors	Shoot length (cm)	Root length (cm)	Seedling fresh weight (mg/plant)	Seedling dry weight (mg/plant)	Root/Shoot ratio
Stress					
Control	3.67^{a}	8.54^{a}	75.7^{a}	4.83^{b}†	2.35
Drought	1.04^{b}	2.71^{b}	48.1^{b}	15.80^{a}	2.29
Treatment					
Unprimed	2.48	3.75^{d}	43.8^{d}	7.21^{d}	1.67^{c}
Hydration	2.41	6.72^{a}	64.3^{b}	10.25^{c}	2.50^{b}
1 ppm B	2.32	5.77^{b}	64.4^{b}	10.85^{abc}	2.39^{b}
10 ppm B	2.20	5.51^{b}	59.9^{c}	10.50^{bc}	2.96^{a}
100 ppm B	2.30	4.43^{c}	64.4^{b}	11.48^{a}	1.83^{c}
500 ppm B	2.33	5.39^{b}	66.8^{ab}	10.63^{bc}	2.54^{ab}
1000 ppm B	2.42	6.51^{a}	69.5^{a}	11.23^{ab}	2.35^{b}
Analysis of Variance					
Stress (A)	**	**	**	**	NS
Treatment (B)	NS	**	**	**	**
A×B	**	**	**	**	**

†: Means followed by the same letter(s) in each column did not significant at $p<0.05$. **: significant at 1%. NS: not significant.

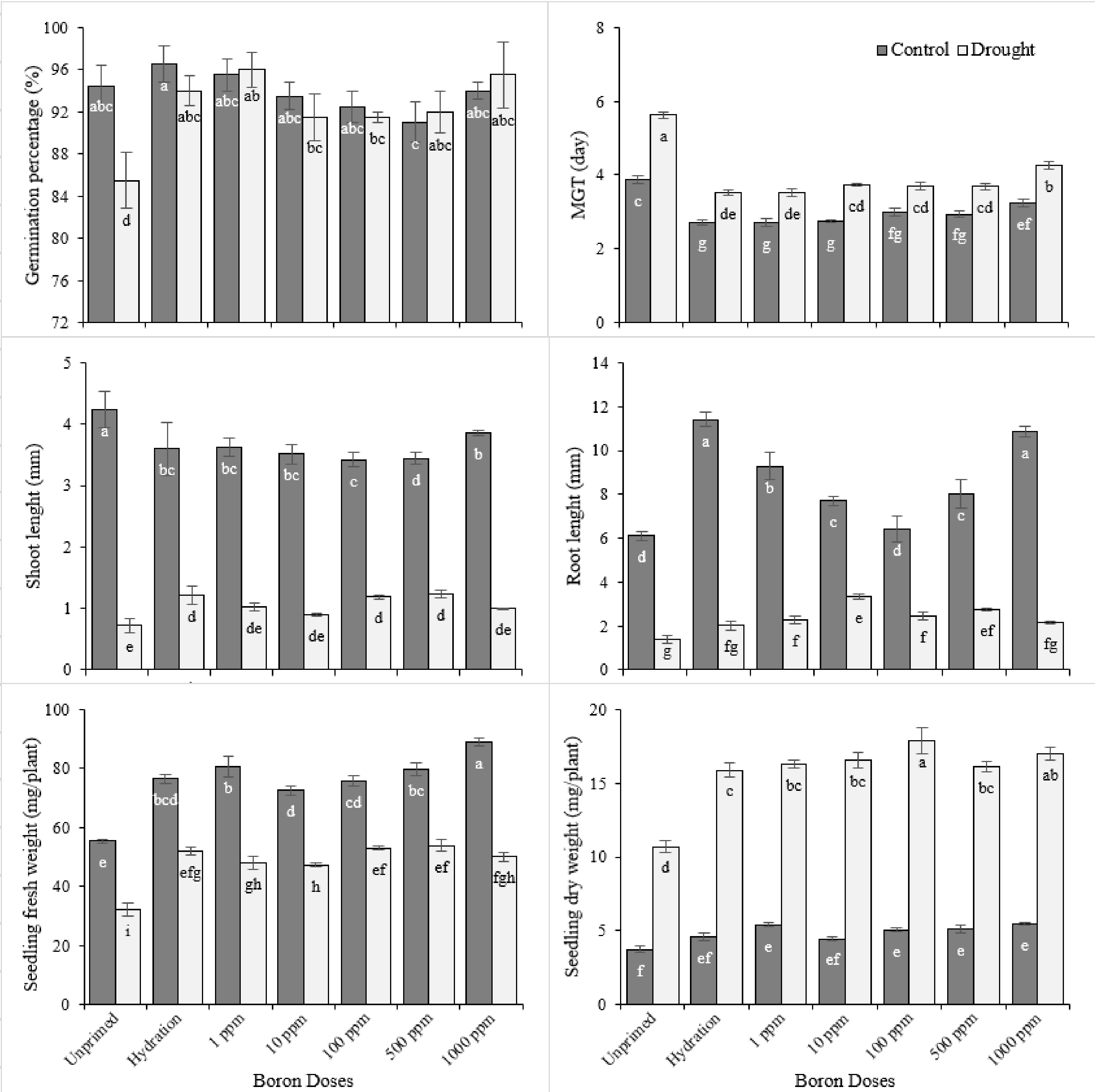

Figure 1. Interaction effects between seed priming with different boron levels and drought stress on germination percentage, mean germination time, shoot length, root length, seedling fresh weight, and seedling dry weight of spinach under control and drought stress.

A two-way interaction showed that priming treatments considerably enhanced the germination percentage of spinach seeds regardless of whether they were subjected to control conditions or drought stress. Unprimed seeds had a lower germination percentage when exposed to drought stress. Memon et al. (2013) found a clear increase in germination percentage of broccoli when its seeds were primed with hydration or 0.01%-0.5% B. Similarly, the beneficial effects of boron priming on germination were reported by Farooq et al. (2011) in rice at a concentration of 0.001-0.01% B and Kaya and Ergin (2023) in safflower at 5 ppm B. However, seeds primed with 1, 500, and 1000 ppm B exhibited a slightly higher germination rate under drought conditions compared to the control group. Drought stress resulted in a longer mean germination time. The highest difference between control and drought was observed in unprimed seeds (Figure 1). Spinach seeds primed with hydration and 1 ppm B had a shorter mean germination time under both control and drought conditions.

The results of Memon et al. (2013) in broccoli, Farooq et al. (2011) in rice, Iqbal et al. (2017) in wheat, and Kaya and Ergin (2023) in safflower support the findings of this study.

Drought stress caused a depression in shoot length of spinach. It was emphasized by the findings of Zargar et al. (2021). Shoot length was statistically decreased by priming treatments in control conditions, but all seed primings showed better shoot growth than unprimed seeds under drought stress (Figure 1). Hydration, 100 ppm B, and 500 ppm B resulted in longer shoots than the others. Similar results were reported by Farooq et al. (2011), Memon et al. (2013), and Kaya and Ergin (2023).

Boron primings improved the root length of spinach under control and drought stress conditions. The longest root length was determined in seeds treated with 10 ppm B under drought, while 500 ppm B produced longer roots than the others. This result was confirmed by the findings of Farooq et al. (2011), Xia et al. (2019), and Kaya and Ergin (2023), who found that root growth of the investigated plants was promoted by boron priming.

Seedling fresh weight of spinach was stimulated by seed priming treatments under both control and drought stress conditions. Seeds primed with 1000 ppm B produced the heaviest seedlings under control, whereas it was the highest at 100 ppm B and 500 ppm B under drought. This result showed similarity with the findings of Memon et al. (2013) in broccoli, Farooq et al. (2011) in rice, and Kaya and Ergin (2023) in safflower, who determined that boron priming led to an increase in seedling fresh weight.

Seed priming promoted seedling dry weight in spinach, but the superiority of priming was evident under drought stress. Under drought conditions, 100 ppm B produced the highest dry weight. All seed priming treatments had heavier dry weight than unprimed seeds. Farooq et al. (2011) reported that boron priming with 0.001-0.01% B increased the dry weight of rice seedlings. Iqbal et al. (2017) found a significant improvement in seedling dry weight of wheat with a low boron dose (0.5 M) seed priming.

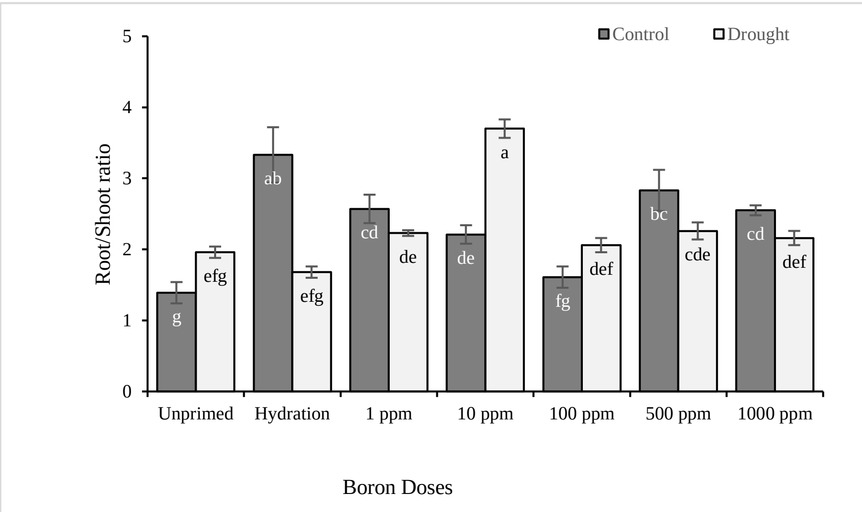

Figure 2. Interaction effects between seed priming with different boron levels and drought stress on root/shoot ratio of spinach.

The root/shoot ratio was changed by boron priming (Figure 2). The maximum ratio was obtained in the seeds primed with 10 ppm B under drought stress. However, the highest ratio in control conditions was recorded in hydration. This finding should be evaluated as boron priming stimulated root growth rather than shoot growth in spinach.

4. Conclusion

Drought is a global phenomenon that adversely affects crop production, and several strategies have been developed to mitigate its negative effects on food supply. Seed priming is a valuable method to improve germination and seedling growth under drought stress after planting. In this study, seed priming with different doses of boron was tested under drought stress in spinach. Boron priming was effective in stimulating germination and seedling growth of spinach. Moreover, seedling growth was induced much more than germination, and root growth was enhanced more than shoot growth by boron priming. This achievement may be due to a reduction in seed-borne pathogens reported by Kaya and Ergin (2023) in safflower. It was concluded that 10-100 ppm B priming should be recommended to increase germination and seedling growth of spinach under both control and drought conditions.

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Conflict of Interest

The author has no conflict of interest to declare.

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