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Research Article

The Boron Application Effects on Germination and Seedling Parameters of Sorghum Cultivars [*Sorghum bicolor* (L.) Moench] in Drought

Tuğba Hasibe GÖKKAYA*[1], Mehmet ARSLAN[2]

[1]Western Mediterranean Agricultural Research Institute, 07100, Antalya, Turkey
[2]Akdeniz University, Faculty of Agriculture, Department of Field Crops, 07070, Antalya, Turkey

[1]https://orcid.org/0000-0001-5956-0764, [2]https://orcid.org/0000-0002-2197-4969

*Corresponding author e-mail: tugbahasibegokkaya@gmail.com

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Abstract: The aim of study was to investigate the effects of boron on germination and seedling parameters of sorghum [*Sorghum bicolor* (L.) Moench] under drought stress conditions. The experiment was conducted in a factorial trial in a randomized plot design with four replication in a growth chamber. In this study, three different sorghum cultivars were used. Drought conditions were performed at three different levels using PEG (0.-0.4 MPa and -0.8 MPa). Four boron doses (0-5-10-15 mM B) solutions are formed as boric acid (H_3BO_3). Parameters measured in Gözde 80 were superior to other cultivars under drought stress conditions. The maximum mean germination time, seedling viability index, shoot and root length, shoot and root fresh weight, and total biomass, were detected as 4 days, 74%, 10 cm, 13 cm, 63 mg, 21 mg, and 80 mg, respectively. Differences were noted in the response of different sorghum cultivars to drought stress, and significant decreases were observed as the drought level increased. Low boron applications generally increased germination and seedling parameters compared to control under drought conditions. The boron effects applied to alleviate the drought stress effects have been noticeably positive. Compared to control conditions, the best results were also generally observed in the application of 5 mM B at a drought stress dose of -0.4 MPa. It was concluded that high doses of boron applications caused double stress with drought and were even lower than drought applications alone. It was recorded that due to the reasons listed above, careful attention should be paid to the boron doses to be applied.

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1. Introduction

Sorghum (*Sorghum bicolor* L.) appears as a product with great potential, therefore it indicates the fifth most considerable grain yield worldwide (Dahlberg et al., 2012; Avila et al., 2021). This plant is a cereal family product with plural uses for food, forage crop, and energy. The feed quality and energy stature of sorghum are similar to maize. Furthermore, sorghum is valued as silage, direct grazing, and fresh and dry fodder (Rad et al., 2021). Sorghum has high genetic variability and germplasm sources to settle new cultivars into varied ecoregions (Qadir et al., 2015; Al-Naggar et al., 2018; Erdurmuş et al., 2021).

Sorghum is a model plant for a more suitable crop development program in agriculture to use marginal areas to supply the energy and food requirements that may increase in the immediate future (Bibi et al., 2012; Qadir et al., 2015). Compared to other cereal crops, sorghum is extremely tolerant of abiotic stresses such as drought, flooding, and salinity. (Ali et al., 2011; Turner et al., 2016). The plants' response to abiotic stress is attached to genetic and environmental conditions. Drought stress reduces sorghum production, and deterioration is more vigorous when lack of water consists of the pre-flowering stage of development (Kothari et al., 2020).

It is estimated that the world population, which was about seven billion in recent years, will reach nine billion by 2050, and to support such a population, food security is more threatened by different kinds of environmental stresses (Khan et al., 2020). In recent years, with the effect of global warming, being an environmental problem, the importance of water has begun to be felt with agricultural drought (Khanna-Chopra and Selote, 2017; Rafique et al., 2022).

Plants grow tolerance mechanisms that provide physiological, biochemical, and molecular responses to drought stress and adapt to environmental conditions (Mut et al., 2010; Franks, 2011). Due to the sensitivity of plant species to drought, which can affect physiological processes such as photosynthesis, nutrient uptake, and transport during the growth phase is water restriction (Cai et al., 2020). Effect of drought stress on plant growth (Yao et al., 2009; Kamran et al., 2018) and yield; It varies depending on the developmental period in which the stress occurs and the severity and duration of the stress (Aykanat et al., 2009). Polyethylene glycol 6000 is the best chemical known for applying water stress, reflecting the kind of stress seen in drying soil (Verlues and Bray, 2004). Gholami et al. (2021) reported that the germination percentage and germination rate tended to decrease with increasing drought stress induced by PEG.

Boron (B) is an essential microelement and is constantly requisitioned by vascular plants to compose varied basic physiological processes in the life cycle, such as carbohydrates and RNA metabolisms (Tanaka and Fujiwara, 2008), the cell wall integrity and plasma membrane, meristematic tissues elongation (Herrera-Rodriguez et al., 2010), the growth and germination of the pollen tube, the flowers fertility formation, anther (Parry et al., 2016) and seed improvement (Iqbal et al., 2017; Nadeem et al., 2019).

Lack of boron can therefore affect and reduce vigor against abiotic stresses such as drought (Möttönen et al., 2001). Nevertheless, a few research has identified the availability of B in drought tolerance in plants. Considering these issues, the aim of this study is to investigate the effects of different boron doses on the germination and seedling growth of sorghum [*Sorghum bicolor* (L.) Moench] under drought stress conditions.

2. Material and Methods

The aim of this experiment is to investigate the curative effects of different boron doses on the germination and seedling growth of sorghum [*Sorghum bicolor* (L.) Moench] plant under drought stress conditions. This experiment was conducted at the forage crops laboratory, Department of Field Crops, Akdeniz University, Turkey during the spring of 2022. The experiment was carried out in four replications with the factorial arrangement according to the randomized plot design. The sorghum seeds were provided from Bati Akdeniz Agricultural Research Institute. Three selected cultivars, Erdurmuş, Uzun, and Gözde 80, were used as genetic materials. Petri dishes used were sterilized and incubated as a general protocol procedure. Twenty seeds from each cultivar were chosen and placed in 9 mm petri dishes, two Whatman filter papers were lined in. Tree drought stress levels causing 0, -0.4MPa, and -0.8 MPa were calculated by the equation of Michel and Merril (1973) using PEG 6000 concentration. Boron was applied as H_3BO_3 at 0-5-10-15 mM. 10 ml of solution was used for moistening in each application. Petri dishes were wrapped with parafilm to prevent water loss by evaporation. The petri dishes were settled in a growth chamber at 20ºC under photoperiodic conditions of 16 hours light and 8 hours dark. Observations were recorded daily. The study ended on the eleventh day.

Germination tests were carried out according to ISTA rules (2017). The seed of germination (MGT) was calculated using formulas described by Majda et al. (2019). The germination rate (GR) was calculated according to Xia et al. (2019). The germination index (GI) and seedling vigor index (SVI) were counted by the method of Xia et al. (2019). The root/shoot ratio (R/S ratio) was calculated as the following equation (Shtaya et al., 2021). The calculation of stress tolerance indices formulas as described by Nawaz et al. (2014).

$$\text{MGT (day)}=\sum \frac{number\ of\ seeds\ germinated\ on\ the\ i^{th}\ day}{number\ of\ days\ to\ count\ the\ n^{th} day} \quad (1)$$

$$\text{GR(\%)}=\frac{\text{number of germinated seed/}}{\text{total number of seeds tested}}*100 \quad (2)$$

$$\text{GI}=\sum \frac{\text{the number of germinated seeds in day}}{\text{day of counting seed germination}} \quad (3)$$

$$\text{SVI}=\frac{\text{germination percentage}*\text{average seedling length}}{100} \quad (4)$$

$$\text{R/S ratio}=\frac{\text{roots length}}{\text{shoot length}} \quad (5)$$

$$\text{SLSI (\%)}=\frac{\text{Shoot lenght of stressed plant}}{\text{shoot length of control}}*100 \quad (6)$$

$$\text{RLSI (\%)}=\frac{\text{Root lenght of stressed plant}}{\text{root length of control}}*100 \quad (7)$$

$$\text{SFSI (\%)}=\frac{\text{Shoot fresh weight of stressed plant}}{\text{Shoot fresh weight of control}}*100 \quad (8)$$

$$\text{RFSI (\%)}=\frac{\text{Root fresh weight stress of stressed plant}}{\text{Root fresh weight of control}}*100 \quad (9)$$

Data determined for the study subjected to analysis variance using R (ANOVA) and means were compared by one-way ANOVA and Duncan's post hoc test in the agricolae, which differed significantly at 0.05 levels. (4.3.19) package.

3. Results and Discussion

Based on the statistical analysis, the plant growth parameters of sorghum cultivars influenced by different boron applications and drought conditions were given in Table 1 and Table 2.

Table 1. Results of variance analysis on growth parameters of boron doses and drought stress levels in sorghum cultivars

SV	df	MGT	GR	GI	SVI	SL	RL	R/S
C	2	0.08	100.52**	738.33**	1 708.97**	28.91**	38.42**	0.03**
DL	2	0.90**	1 338.02**	1 575.97**	595.85**	28.28**	23.77**	0.01
B	3	0.36**	2 000.93**	2 280.07**	881.45**	131.34**	223.31**	0.62**
C*DL	4	0.11*	65.10**	70.50**	45.25**	4.58**	0.97*	0.07**
C*B	6	0.02	83.39**	85.27**	58.48**	0.93**	3.75**	0.05**
DL*B	6	0.04	66.03**	63.51**	28.21**	1.81**	8.05**	0.08**
C*DL*B	12	0.06	8.39	10.08	3.95	0.83**	2.14**	0.04**

*Significant at the 0.05 probability level.**Significant at the 0.01 probability level. (Cultivar: C, Drought level: DL, Boron: B, Mean germination time: MGT, Germination Rate: GR, Germination index: GI, Seedling Vigor index: SVI, Shoot length: SL, Root length: RL, Root/shoot rate:R/S)

Except for mean MGT and RFSI, the growth parameters, studied in the experiment, were significantly (p<0.01) influenced in each cultivar. Similarly, drought conditions adversely affected the seedling growth parameters except for the R/L rate (p<0.01). Increasing boron application caused a significant (p<0.01) effect on parameters. MGT, RL, and RFW were statistically influenced by cultivar and drought interaction (p<0.05) and the others were also affected by interaction, significantly except RFSI. The interaction of cultivar, drought, and boron applications had a meaningful effect (p<0.01) on the plant growth parameters except for MGT (Table 1 and Table 2).

Table 2. Results of variance analysis on growth parameters and stress tolerances of boron doses and drought stress levels in sorghum cultivars

SV	df	SFW	RFW	TB	SLSI	RLSI	SFSI	RFSI
C	2	166**	163.81**	657.10**	3063**	913**	4573.00**	65
DL	2	1 179**	81.92 **	1 872.90**	113 971**	127 135**	116 172**	110 496**
B	3	4 999**	540.10**	8 792.70**	567**	1 588**	973.00**	5 204**
C*DL	4	260**	4.82*	205.80**	786**	260.00**	1 218**	57.00
C*B	6	75**	39.53**	184.20**	361**	1 104**	864**	1 423.00**
DL*B	6	99**	28.09**	223.10**	200**	578**	293**	1 658.00**
C*DL*B	12	57**	7.97**	64.50**	129**	516.**	297**	524.00**

*Significant at the 0.05 probability level.**Significant at the 0.01 probability level. (Cultivar: C, Drought level: DL, Boron: B, Mean germination time: MGT, Germination Rate: GR, Germination index: GI, Seedling Vigor index: SVI, Shoot length: SL, Root length: RL, Root/shoot rate:R/S)

Generally, the maximum growth parameters were realized with Gözde 80. Nevertheless, the MGT and GI were determined at the highest level in Erdurmuş. When the results were examined on the basis of cultivars, MGT was found as 3.74, 3.71, and 3.65 days at Uzun, Gözde, and Erdurmuş, respectively. A higher germination time indicates late emergence. The TB was observed as 40.60, 46.02, and 47.76 mg with Erdurmuş, Uzun, and Gözde 80, respectively (Table 3). In this study, the different botanical characteristics caused the differences in the effect of drought conditions (Ulukapi & Nasircilar, 2021) and boron doses. On the other hand, it was thought that some cultivars grown enriched with boron and drought conditions have developed resistance to stress.

Table 3. The effects of boron doses on growth parameters of sorghum cultivars exposed to drought stress level

Growth Parameters	Erdurmuş	Uzun	Gözde
MGT (day)	3.65±0.23b	3.74±0.24a	3.71±0.25ab
GR (%)	89.17±7.32a	86.77±7.89b	86.56±10.78b
GI (%)	96.40±7.91a	95.09±8.64b	89.05±11.21c
SVI (%)	52.16±4.28c	55.01±5.00b	63.62±7.92a
SL (cm)	5.82±2.04c	6.34±1.84b	7.35±1.81a
RL (cm)	5.01±2.02c	5.91±2.61b	6.79±2.67a
R/S	0.85±0.15b	0.89±0.19a	0.90±0.15a
SFW (mg)	35.21±12.43b	38.07±13.40a	38.70±10.52a
RFW (mg)	5.45±2.02c	7.95±4.79b	9.06±5.06a
TB (mg)	40.60±14.19c	46.02±17.86b	47.76±15.12a

Different letters next to values indicate statistically different means at p<0.05 level, and p<0.01 levels.

In this experiment, the lowest growth parameters were obtained in -0.8 Mpa DL and the highest were achieved in non-DL. The drought levels increased as the growth declined. The SL was determined as 7.33, 6.40, and 5.80 cm at increasing DL, respectively (Table 4). Drought stress conditions reduce plant growth (Gökkaya, 2016; Gulser et al., 2019) and cell division (Farah, 1981). As a result, this situation leads to the shrinking organs of plants proportionally. As it is known, if the plant height is getting higher, it increases surface evaporation and also the loss of water. Similarly, several researchers reported that drought stress has inhibitory effects on plant growth and decreases the shoot and root fresh weight (Sattar et al., 2021, Rafique et al., 2022).

Table 4. The growth parameters of sorghum cultivars exposed to drought stress levels

Growth Parameters	0 MPa	-0.4 MPa	-0.8 MPa
MGT (day)	3.57±0.19c	3.69±0.22b	3.84±0.23a
GR (%)	92.50±6.01a	88.02±7.20b	81.98±9.55c
GI (%)	99.01±6.75a	93.96±7.94b	87.57±10.90c
SVI (%)	60.23±7.01a	57.34±7.53b	53.22±6.90c
SL (cm)	7.33±211a	6.40±1.77b	5.80±1.81c
RL (cm)	6.66±3.11a	5.78±2.33b	5.27±1.87c
R/S	0.87±0.21	0.88±0.17	0.90±0.11
SFW (mg)	42.41±13.83a	37.09±10.66b	32.50±9.83c
RFW (mg)	8.92±5.70a	7.16±3.92b	6.37±2.85c
TB (mg)	51.33±18.37a	44.24±14.30b	38.87±12.44c

Different letters next to values indicate statistically different means at $p<0.05$ level, and $p<0.01$ levels.

Water deficit decreased seed GR and seed germination percentage. Germination can be affected by water absorption of seeds at strongly negative water potentials, especially at the inhibition beginning, and this event cannot reverse the germination process. Osmotic pressure and increased PEG concentration, cause a decrease in water potential, inhibiting water absorption, delaying germination time, and reducing the germination rate as reduced absorption may inhibit the germination metabolic process (Jamil et al., 2006, Dawadi et al., 2019). Similar to previous studies, it was determined that PEG-induced drought stress was negatively associated with germination (Khodarahmpour, 2011).

Some effects of drought stress on plant growth are enhanced energy demand due to an increase in plant respiration (Moud and Maghsoudi, 2008) and a decrease in the level of photosynthesis (Abdel-Motagally and El-Zohri, 2016). This causes an increase in the stiffness of the cell wall, reducing the rate of cell division, expansion, and elongation (Baek et al., 2005, Sadak et al., 2020). RL and SL and SFW decreased with increasing PEG levels (Bilgili et al., 2019). As a result, seedling growth was inhibited in sorghum (Table 4).

Plant roots hold a key role in uptaking the water and nutrients (Wu et al., 2019). Various studies have shown that root water uptake is related to root morphology (Gao et al., 2010; Yang et al., 2012; Li et al., 2015). In addition, the development of plants is one of the criteria for evaluating drought tolerance and is directly affected by the degree of root development (Chen et al., 2011). Many researchers have reported that plant roots exposed to a lack of water develop better than roots that grow without water restriction (Almaghrabi, 2012; Liu et al., 2017). However, in our study, increasing PEG levels decreased the RL. The increase in RL appears to be due to uptake from the water source, and we experienced drought stress in aqueous solutions of PEG in our study, so both cultivars had reduced roots (Table 4).

Results obtained in our experiment, TB showed significantly decreased under drought stress in cultivars (Table 4). These results are the same those determined by Sadiq et al. (2018) and Dawood et al. (2019). The drought's adverse effects on fresh shoot biomass may be due to reduced photosynthesis rates under drought conditions (Haq et al., 2014).

Table 5. The effects of boron doses on growth parameters of sorghum cultivars

Growth Parameters	0 mM	5 mM	10 mM	15 mM
MGT (day)	3.66±0.25b	3.62±0.19b	3.67±0.22b	3.85±0.23a
GR (%)	89.86±3.04b	95.14±4.70a	87.64±6.03c	77.36±8.22d
GI (%)	96.28±4.19b	101.57±5.83a	93.52±6.34c	82.68±10.40d
SVI (%)	58.50±5.06b	61.92±5.53a	57.16±5.00c	50.15±5.74d
SL (cm)	7.24±1.08b	8.59±1.47a	6.13±0.90c	4.08±0.92d
RL (cm)	6.99±1.46b	8.72±1.97a	4.91±0.80c	2.99±0.79d
R/S	0.96±0.11b	1.02±0.16a	0.81±0.12c	0.74±0.10d
SFW (mg)	41.12±7.31b	51.13±8.99a	33.88±5.02c	23.19±4.35d
RFW (mg)	8.55±2.83b	12.35±4.88a	5.65±1.15c	3.38±0.78d
TB (mg)	49.67±9.22b	63.48±12.42a	39.53±5.20c	26.57±4.83d

Different letters next to values indicate statistically different means at $p<0.05$ level, and $p<0.01$ levels.

The highest means were reported at 5 mM boron level. It was indicated that low boron increases the parameters, moreover, the others decrease the growth (Table 5). Also, Muhammad et al. (2013) and Habtamu et al. (2014) determined that high boron doses decreased the germination percentage. These results correlate with our study. The GI of seeds was directly obtained by boron levels like other properties (Xia et al., 2019). The highest MGT was realized at 15 Mm B with 3.85 days (Table 5). Memon et al. (2013) and Xia et al. (2019) have similar results. Excessive boron doses could not provide sufficient energy for seeds in time to complete their germination and seedling growth (Deb et al., 2010; Chen and Arora, 2013; Iqbal et al., 2017). Low concentration of boron had positive effects on meristematic growth (Khan et al., 2006) and SVI (Table 5, Farooq, 2011; Xia et al, 2019). The SVI frequently reflected the establishment ability of the seedling during plant growth (Xia et al., 2020) In this experiment SL was reduced by 52.50 % and RL decreased by 65.71 % (Table 5). The main reason for the reduction in RL is that higher doses of boron inhibit root growth (Habtamu et al., 2014), primarily by limiting cell protraction and cell division (Brown et al., 2002). Increasing boron doses above 5 mM significantly reduced the FW of plants (Ayvaz et al., 2012; Mohammed et al. 2013; Habtamu et al., 2014). Compared to the control application, there was a 27.80% increase in TB at the 5 mM boron, whereas higher doses caused 20.41% and 46.50% reduction, respectively (Table 5).

Figure 1. Effects of boron doses on SLSI, RLSI, SFSI, and RFSI of sorghum cultivars under drought conditions (E: Erdurmuş, U: Uzun, G: Gözde 80, D1: -0.4 MPa, D2:-0.8 MPa, B0: 0 mM B, B1 5 mM B, B2: 10 mM B, B3: 15 mM B, Shoot length stress tolerance index: SLSI, Root length stress tolerance index: RLSI, Shoot fresh weight stress tolerance index: SFSI, Root fresh weight stress tolerance index: RFSI).

When it was noticed cultivar x drought x boron interactions, the lowest and highest SL and SI have obtained as 59.79 % and 116.64 % in -0.8 MPa and 10 mM B application in Erdurmuş and -0.4 MPa and 10 mM B application in Uzun, respectively. While the lowest RLSI was determined as 57.10 % (Erdurmuş) in -0.8 MPa-0 Mm B application, the highest was in -0.8 MPa-10 Mm B application at Uzun (115 %). The highest SFSI was found as 131.07 % by -0.4 MPa- 10 mM B application in Uzun. The lowest was detected as 59.96 % (Erdurmuş) in -0.8 MPa- 0mM B applications. The lowest and highest RFSI belong Gözde 80 were realized as 52.15 % and 133.52 % in -0.8 MPa 0 Mm B and 10 Mm

B applications, respectively (Figure 1). The process of cell wall synthesis and elongation in plants is highly sensitive to water restriction, and the reduction in growth may be due to decreased turgor pressure of cells (Mohammadkhani and Heidari, 2008). In this study, it was observed that drought levels decreased sorghum plant growth. But low boron applications had significant and positive effects on stress tolerances.

Conclusion

As a result of this study, a decrease in germination and seedling parameters was obtained at different drought levels created with PEG. It has been observed that different species give different responses. In addition, it was determined that low-level boron applications caused an increase in these parameters in drought conditions created with PEG. The results of different drought levels determined in this study and the effects of boron applications on germination and seedling are thought to be useful for further studies.

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