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Effects of PEG-Induced Drought Stress and Different Boron Levels on Seed Germination and Seedling Growth Characteristics in Chickpea (*Cicer arietinum* L.) and Lentil (*Lens culinaris* Medik.)

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Abstract: In the present study, the drought tolerance potential of chickpea (*Cicer arietinum* L.) and lentil (*Lens culinaris* Medik.) seeds under different boron (B) levels were assessed. One chickpea (Azkan) and one lentil cultivar (Sahan) were selected for the genetic material. To provide drought condition, different level of polyethylene glycol solution (PEG 6000) was applied to seeds. Germination experiments were performed under PEG-induced stress to create water potentials of 0 (control), -2, and -4 MPa. Then, boron was applied as H_3BO_3 at 0 (control), 5, and 10 mM. The effects of these abiotic stresses were determined with the measurement parameters of germination rate and root traits. Drought stress adversely affected germination rate and seedling growth characteristics in chickpea and lentil. Especially, seed germination rate is extremely reduced by increased levels of drought stress. An increase in PEG levels from 0 to -4 MPa drastically decreased root and shoot width, and shoot length in chickpea whereas they did not generate a significant difference in seedling growth traits except for root width in lentil. Additionally, the results showed that increasing B treatments decreased the germination rate in both chickpea and lentil. The low concentration of B (5 mM) increased root and shoot length; however, a remarkable decrease was observed in root and shoot growth traits at the highest concentration of B (10 mM). The overall findings show that germination and seedling growth parameters were greatly inhibited by different concentrations of PEG and > 10 mM B levels for chickpea and lentil production.

Keywords: Abiotic stress, boron treatment, germination, legume, PEG 6000

1. Introduction

The legume family (Fabaceae or Leguminosae) is the third largest family of angiosperms. This family represents more than 750 genera and 22.000 species including grain, pasture, and economically important legumes (le Roux et al., 2022). Grain legumes contain an important source of dietary protein (20-50%), especially of amino acid lysine which is generally deficient in cereals (Maphosa and Jideani, 2017). They also include complex carbohydrates and micronutrients such as iron, potassium, and zinc as well as vitamins A and B in addition to folate and thiamine (Gaur et al., 2016) and have potential protective beneficials for cancer (Messina, 1999), diabetes, and obesity (Burstin et al., 2011). Due to their capacity for biological fixation of atmospheric nitrogen through nodulation with *Rhizobium* species, the legumes have an important part in crop rotation (Herridge et al., 1993). The legumes include cool-season legumes such as chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* Medik.), and faba bean, and warm-season legumes such as common bean, pea, cowpea, lupins, soybean, and peanut. Chickpeas and lentils are one of the most important legume crops especially grown in arid and semi-arid areas in the world (Mohammed et al., 2017; Zeroual et al., 2023). The chickpea is the third most important legume in terms of global production after the common bean and soybean, with over 15.9 million tonnes produced in 2022. Nearly 5.6 million tonnes of lentils were produced globally in 2022, fifth among pulse crops after common beans, chickpeas, dry peas, and cowpeas (Anonymous, 2022). However, abiotic stresses such as drought, extreme temperatures (heat and cold), waterlogging, soil salinity, and nutrient deficiencies or toxicities are important limiting factors of chickpea and lentil production worldwide (Siddique et al., 2000; Toker et al., 2007; Sabagh et al., 2021).

Drought stress is the most important abiotic stress that reduces yields in chickpea and lentil since they are mostly grown in arid/semi-arid areas under rainfed conditions (Toker et al., 2007; Toker and Yadav, 2010; Idrissi et al., 2015). Drought stress destroys many physiological and biochemical processes related to cellular processes and photosynthesis (Chaves et al., 2009; Pinheiro and Chaves, 2011; Upadhyaya et al., 2012; Akter et al., 2021), and has a negative effect to N_2 fixation, resulting in significant yield losses (Thudi et al., 2014; Venugopalan et al., 2021). In addition, drought can occur with other stress factors at the same time, such as heat stress, which further leads to reproductive losses (Choukri et al., 2020; El Haddad et al., 2020).

Micronutrients are essential for increasing the yield of pulses due to their effects on the process of symbiotic N_2 fixation. Boron (B) is an important micronutrient and has a significant role in cell division, carbohydrate metabolism, elongation in meristematic tissues and floral organs, pod/seed formation, yield and yield components (Marschner, 1995; Dell et al., 1997; Özyazıcı and Açıkbaş, 2021). Thus, the requirement of B seems more important in the reproductive growth period (Nalini and Bhavana, 2013). In chickpeas, B deficiency causes flower drops resulting in low pod sets and seed yields (Valenciano et al., 2010). The lack of B similarly leads to a decrease in yield in lentil-growing areas (Oktem, 2022). However, excessive intake of B results in B toxicity that restricts plant growth and damages the photosynthetic system (Riaz et al., 2021). Ardıc et al. (2009) showed the effects of B toxicity on the activities of antioxidant enzymes in chickpea. Besides, Tepe and Aydemir (2017) mentioned the toxic effects of B on plant growth and the antioxidant response of lentil cultivars.

Abiotic stress factors may affect plants at all stages of growth and development (Shabbir et al., 2022). However, the plants are more sensitive to abiotic stress during early seed and seedling stages (Cuartero et al., 2006). So, these stages are extremely critical for initial growth and getting an optimal number of seedlings for higher seed yield. Many studies indicated that different B levels (Valenciano et al., 2010; Hoque et al., 2021; Iqbal et al., 2022; Oktem, 2022) and drought (Saglam et al., 2014; Khatun et al., 2021) influenced the seed germination performance and seedling traits in chickpea and lentil. In these studies, different B levels were selected to understand the effects of drought. In addition, osmotic adjustment plays an important role in tolerance to drought during germination (Muscolo et al., 2014). Polyethylene glycol (PEG), is a non-ionic surfactant, that has the capability of inducing water stress (Larher et al., 1993) and is mainly used to regulate water availability in seed germination (Kulkarni and Deshpande, 2007). With all this, this study aimed to investigate the effects of drought and boron stresses on the growth and physiological properties of chickpea (*C. arietinum*) and lentil (*L. culinaris*).

2. Materials and Methods

Chickpea (*C. arietinum*) seeds of the released cultivar, Azkan, and lentil (*L. culinaris*) seeds of the released cultivar, Sahan were used as a plant material. The experiment was carried out in the Forage Crops Laboratory of Akdeniz University, Antalya-Türkiye. The seeds were sterilized and after that rinsed with sterile water. Four replications of the seeds (5 chickpea seeds and 5 lentil seeds) were placed on 9-cm Petri dishes having one layer of Whatman No. 1 filter paper. The seeds were soaked in different concentrations of PEG 6000 (Polyethylene glycol 6000) solution as 0 (control), -2, and -4 MPa. Boric acid (H_3BO_3) was used as a B source at different levels (0.5, and 10 mM). The Petri dishes were moistened with either deionized water as a control option or 10 ml of treatment solution for each application and they were incubated in 16 h light and 8 h dark photoperiod for 10 days at 20 °C and 70% relative humidity. Germination counts were made each 3 days when their shoot length was at least 1 mm long. Germination rate was calculated with the formula as seeds germinated/total seeds x 100. After 10 days of seed germination, data for root length, shoot length, root width, shoot width, root fresh weight, and shoot fresh weight were measured and subjected to analysis of variance (ANOVA) and the least significant difference (LSD) test for comparisons using SAS version 9.3 (Anonymous, 2011).

3. Results

Drought stress primarily affected germination rate, root width, shoot length, and shoot width in chickpea whereas it influenced germination rate and root width in lentil (Table 1). Different rates of PEG negatively affected the germination rate in chickpea. A reduction of up to 8.4 and 26.7% germination rate was monitored when the use of -2 and -4 MPa compared to the control treatment (96.7%), respectively. Increasing PEG concentrations from 0 to -4 MPa drastically decreased root width and the lowest value was recorded at -4 MPa. The root width was found as 2.5, 2.1, and 1.9 cm at 0 (control), -2, and -4, respectively. Shoot length significantly decreased from -2 to -4 MPa. In the control condition, the longest shoot length was 2.6 cm. There was a significant reduction in shoot width with increasing concentrations of PEG. The highest value for shoot width as 2.4 cm was observed in the control condition. The fresh weight of root and shoot also declined with increasing PEG levels, however, there was no significant difference. In lentil, germination ratios declined from the control condition to -4 MPa (Table 2). A significant reduction was observed in germination ratio with increasing concentrations of PEG from 2 to -4 MPa. Different drought levels did not generate a significant difference in seedling growth traits except for root width. The highest value was 0.9 cm in the control condition. It was also observed as 0.8 with the same values at -2 and -4 MPa.

The ANOVA analysis revealed that boron significantly influenced root width and root fresh weight, but it did not significantly influence the germination rate in chickpea. It also did not affect all growth traits except for root and shoot fresh weights. Additionally, there was a significant interaction among drought and boron stress only in root fresh weight. The boron treatments reduced the germination rate compared to control, however, there was no statistical difference. The highest value was observed in the control (88.3%). It declined with increasing levels of boron. Significant differences were observed among boron treatments in root width, shoot width, and root fresh weight. 5 and 10 mM concentrations made a strong suppression of root width. The highest root width was recorded in the control (2.4 cm) and the lowest was measured in the concentration of 10 mM (2.0 cm). The highest root fresh weight was observed at 5 mM concentration with 0.5 cm compared to the control. In lentil, different boron levels significantly influenced all seedling growth traits however there is no statistically significant difference in the trait of germination rate. The highest value was 96.7% under control conditions. An increase from 5 to 10 mM did not affect the germination value in lentils. Increasing boron levels reduced root length drastically in the seeds. The lowest value was observed in the concentration of 10 mM boron. The root width also decreased with the increase in the boron level, significantly. Lower values were observed in the high concentrations of boron. 5 mM boron treatment provided the highest values in root length, shoot length, shoot width, and root fresh weight compared to the control treatment (Table 3). In terms of shoot fresh weight, the same values (0.9 cm) were observed in all boron levels. However, there was not a statistically significant difference.

4. Discussion and Conclusion

In the present study, germination traits were negatively affected by drought stress in chickpea. Especially, the increase of drought conditions drastically reduced the seed germination rate (Table 2). Correlative results were obtained by Koskosidis et al. (2020) and Yucel et al. (2010) who indicated drought negative effect on the germination of chickpea seeds. The same negative effects on germination ratio were also observed in lentil. Previously, Foti et al. (2018) and Muscolo et al. (2014) reported that drought stress reduced germination percentage by 20% in lentil. The decrease in germination of the seed might be caused by lower water uptake through the testa (Bahrami et al., 2012). Metabolic changes that occurred during germination might also lead to lower success rates (Ayaz et al., 2000). Root traits are valuable features because they improve crop yield under drought stress (Ye et al., 2018). Considerable advances have been made in clarifying the role of the root traits for drought stress tolerance in chickpea (Krishnamurthy et al., 2003; Chen et al., 2017). In the present study, only root width was negatively affected by drought conditions. An increase was observed in root length. Increasing the capacity of root systems was previously reported in rice (Courtois et al., 2009), wheat (Sharma et al., 2011), and maize (Giuliani et al., 2005). Increased root biomass, root length, and root weight are often considered to be primary strategies for drought stress avoidance (Kashiwagi et al., 2005). In chickpea, a significant decrease in shoot length and shoot width was observed at higher PEG levels. However, it did not negatively affect these traits in lentil seeds although the results of Foti et al. (2018) were contrary to ours. Although Mujtaba et al. (2016) reported that low water levels restricted plant growth which could potentially lead to decreasing in biomass, an increase in drought stress level did not cause a decline in root and shoot fresh weights of chickpea and lentil. Correlative result was obtained by Kashiwagi et al. (2005). The germination rate was reduced with the increase of boron treatments for both species. The highest germination rate was monitored in the control (88.3%). It declined up to 80% with increasing levels of boron in chickpea. The decrease was from 96.7% (control) to 95% (10 mM) in lentil. The results showed that increasing boron treatments reduced to germination rate in both chickpea and lentil. This was in accordance with the findings of Shah et al. (2013) and Alamri et al. (2018) who

Table 1. ANOVA on mean of squares of measured traits in chickpea and lentil under control and different levels of PEG and B

Source of variation	df	Germination rate (%)	Root length (cm)	Root width (cm)	Shoot length (cm)	Shoot width (cm)	Root fresh weight (g)	Shoot fresh weight (g)
				chickpea				
Drought	2	2233.3*	2.0^{ns}	1.3**	2.9*	0.4*	0.1^{ns}	0.1^{ns}
Boron	2	233.3^{ns}	6.4^{ns}	0.4*	0.6^{ns}	0.2^{ns}	0.4*	0.1^{ns}
Drought x Boron	4	66.7^{ns}	0.6^{ns}	0.1^{ns}	0.1^{ns}	0.1^{ns}	0.2^{ns}	0.1^{ns}
				lentil				
Drought	2	277.77^{ns}	4.26^{ns}	0.05^{ns}	0.01^{ns}	0.02^{ns}	0.01^{ns}	0.01^{ns}
Boron	2	11.11^{ns}	52.38**	0.05^{ns}	1.52**	0.05^{ns}	0.03**	0.01*
Drought x Boron	4	27.77^{ns}	0.25^{ns}	0.01^{ns}	0.26^{ns}	0.03^{ns}	0.02**	0.01^{ns}

ns, * and ** represent non significant, significant at 5% and 1% probability levels, respectively; df, represents degree of freedom

Table 2. Mean comparison of main effects of PEG in chickpea and lentil*

Drought stress level (bar)	Germination rate (%)	Root length (cm)	Root width (cm)	Shoot length (cm)	Shoot width (cm)	Root fresh weight (g)	Shoot fresh weight (g)
			chickpea				
0	96.7 a	3.9	2.5 a	2.6 a	2.4 a	0.6	0.5
2	88.3 a	4.6	2.1 b	2.5 a	2.2 b	0.5	0.5
4	70.0 b	4.7	1.9 c	1.7 b	2.1 b	0.5	0.4
			lentil				
0	98.3 a	5.0	0.9 a	2.0	1.0	0.1	0.1
2	92.9 b	5.2	0.8 b	2.1	1.0	0.1	0.1
4	73.4.0 c	6.0	0.8 ab	2.1	1.0	0.1	0.1

*: Mean with different letter(s) in each trait is significantly different according to LSD multiple range test.

Table 3. Mean comparison of main effects of B levels in chickpea and lentil*

Boron level (mM)	Germination rate (%)	Root length (cm)	Root width (cm)	Shoot length (cm)	Shoot width (cm)	Root fresh weight (g)	Shoot fresh weight (g)
			chickpea				
0	88.3	4.2	2.4 a	2.4	2.3 a	0.8 a	0.5
5	86.7	5.2	2.1 b	2.5	2.1 b	0.9 a	0.6
10	80.0	3.8	2.0 b	2.0	2.0 b	0.4 b	0.4
			lentil				
0	96.7	6.1 a	0.9 a	2.2 a	0.9 b	0.1 b	0.1 ab
5	95.0	7.1 a	0.8 ab	2.3 a	1.0 a	0.2 a	0.1 a
10	95.0	3.1 b	0.7 b	1.6 b	0.9 ab	0.1 ab	0.1 b

*: Mean with different letter(s) in each trait is significantly different according to LSD range test.

identified negative effects of high dose boron on maize and barley, respectively. Turhan and Kuscu (2021) reported that the decline in germination ratio started with 2.0 mg L^{-1} boron and upper levels. They also observed that the germination ratio was 51.89% in pepper, 50.18% in eggplant, and 53.06% in watermelon at 16.0 mg L^{-1} of boron concentration. The most visible change of boron deficiency reducing root growth, after exposure to B-deficient conditions (Marschner, 2012). The low concentration of boron (5 mM) increased root and shoot length in chickpea and lentil. Camacho-Cristóbal et al. (2015) mentioned that B deficiency negatively affects cell elongation of the primary root. However, excess boron primarily inhibits plant germination and cell division and damages the thylakoid assembly by affecting photosynthesis, thereby reducing CO_2 absorption, and resulting in reduced root and shoot growth (Reguera et al., 2009). This hypothesis was in concordance with our results since a remarkable decrease was observed in root and shoot growth traits at the highest concentration of boron (10 mM). Similar results were reported by Valenciano et al. (2010). 5 mM boron concentration increased root and shoot fresh weights in chickpea and lentil. However, they declined under 10 mM concentration. The decline was higher in the chickpea compared to the lentil. So, the lentil could be evaluated as more tolerant to high concentrations of boron. Tepe and Aydemir (2017) also reported 0.5 and 1.0 mM concentrations of boron increased fresh root and shoot weight in lentil. Molassiotis et al. (2006) reported that a 0.5 mM concentration of boron increased dry weight, however, Karabal et al. (2003) observed a decline in root and leaf dry weights of barley under a high concentration of boron (10.0 mM).

Water scarcity is one of the most important agricultural and environmental threats in cultivated areas and based on climatic predictions, the availability of water resources is expected to decrease (Ceccarelli et al., 2010). Drought is a temporary decrease in water availability because of insufficient rainfall that limits plant growth, development, and productivity in many crops worldwide. So, the focus on breeding for drought tolerance mechanisms is becoming increasingly important. The present study investigated the effects of drought on the growth and physiological properties of chickpea and lentil under increasing boron treatments. Drought stress adversely affected to germination rate and seedling growth characteristics in chickpea and lentil. Especially, the seed germination rate is extremely reduced by increased levels of drought stress. The results also showed that increasing boron treatments decreased the germination rate in both chickpea and lentil. As a result, the overall findings show that germination and seedling growth parameters were greatly inhibited by different concentrations of PEG and >10 mM boron levels for chickpea and lentil production.

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Declaration of Conflicts of Interest

No conflict of interest has been declared by the author.

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