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The Impact of PEG-induced Drought Stress on Seed Germination and Initial Seedling Growth of *Lupinus albus* L.

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ABSTRACT

Drought is regarded as one of the most significant abiotic constraints to agricultural crop output worldwide. Drought in the spring and early summer, which coincides with important reproductive stages, severely limits lupin yield in Mediterranean climate zones. The purpose of this study was to determine how different drought treatments affected seed germination and initial seedling growth in *Lupinus albus* L. (white or field lupin). Seed germination parameters and initial seedling growth traits were tested against five levels of drought stress induced with Polyethylene glycol-6000 (PEG$_{6000}$) at concentrations of 0, 4, 8, 12, and 16%. An experiment with four replications was conducted using a completely randomized design. The results revealed that the negative effect of drought stress started at 4% (-0.03 MPa or -0.3 bar) treatment for the initial seedling growth stage; whereas, it started at 12% (-0.2 MPa or -2 bar) treatment for the germination stage. Therefore, it was determined that *L. albus* was more sensitive to drought stress at the initial seedling growth stage than at the germination stage. However, it was observed that the growth parameters were more sensitive in shoot growth than in root growth to drought stress. There will be a sharp loss of yield in soils with levels of drought stress imposed by 12% PEG$_{6000}$ (-0.2 MPa-moderate drought-) and beyond. Therefore, it is likely that *L. albus* has low drought tolerance.

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Introduction

Abiotic stresses such as drought, together with rising global population and per capita food consumption, pose a threat to global food supplies. Drought and other abiotic stressors reduce production and plant growth. They inhibit photosynthesis, limiting the supply of photosynthetic assimilates and energy to the plant. Plants must maximize their utilization of this limited supply of nutrients in order to live under stress (Bibi et al., 2012). Therefore, it is crucial to understand how plants react to drought in order to predict crop germination and establishment under conditions of limited water supply.

Chemical compounds such as polyethylene glycol (PEG), mannitol, sodium chloride (NaCl), sucrose, and glucose are frequently used to imitate osmotic stress during the germination stage (Kouighat et al., 2021). Polyethylene glycol (PEG) molecules with a molecular weight of 6000 are non-ionic, static, and cell-impermeable. They are small enough to impact osmotic pressure, but large enough that they are not absorbed by plants. As a result, they are commonly used. PEG$_{6000}$ is employed in the current work to induce osmotic stress in seedlings (Jamil et al., 2019).

Lupinus albus L. (also known as white or field lupin), a cool-season grain legume, is gaining popularity in European agriculture as a means of increasing dietary protein availability, addressing the severe lack of protein feedstocks, and improving agricultural sustainability (Annicchiarico et al., 2018). The adaptation of white lupin to extreme drought is crucial in Mediterranean climate zones, as this stress coincides with critical reproductive stages. Drought stress is expected to spread throughout the Mediterranean basin and into Europe as a result of reduced rainfall and higher evapotranspiration caused by climate change. The information available on the amount of white lupin genetic diversity for drought resistance is limited and mostly concerns landrace material (Pecetti et al., 2023). Despite all these important features, as in other plants, *Lupinus albus* L. is significantly affected by drought (Slabu et al., 2010). Despite its ability to recover quickly from severe water deficit (Pinheiro et al., 2004), *L. albus* suffers yield losses due to drought in the field, typically ranging from 30% to 90% (Hussain et al. 2019; Dietz et al. 2021). In addition, according to Annicchiarico et al. (2018), drought causes a 79% loss in grain yield in *Lupinus albus* L. Moreover, drought, salt, and mineral fertilizer deficit are major limitations for lupin production (Yu & Rengel 1999). Therefore, it is important to determine the response of white lupine to drought. However, there are a limited number of reports available on the germination and initial seedling growth of *L. albus* under drought stress. Although many species respond to drought conditions, stress thresholds may change depending on genotype (Kayaçetin, 2022a). Studies generally focus on narrow-leafed lupin (*Lupinus angustifolius* L.). Therefore, this study was conducted to test the germination and initial growth parameters of L. albus in different PEG_{6000}-induced drought levels under laboratory conditions.

Materials and Methods

Material

Seeds of *Lupinus albus* L. acquired from RLP AgroScience GmbH (Germany) were used as plant material in the study. This research was conducted at Kırşehir Ahi Evran University (Türkiye), Faculty of Agriculture, Department of Soil Science and Plant Nutrition.

Germination Tests and Morphological Observations

Test solutions were prepared with distilled water at concentrations of 0% (control), 4%, 8%, 12%, and 16% PEG_{6000}. According to Michel and Kaufmann (1973), this osmotic potential equivalent is -0.3 bar (-0.03 MPa), -1.03 bar (-0.1 MPa), -2.01 bar (-0.2 MPa), and -3.3 bar (-0.33 MPa). Four replicates of 25 seeds were germinated between three rolled filter sheets using 10 mL of the relevant test solutions. Before planting, seeds were given a fungicide treatment (Tetramethylthiuram disulphide -80%-), and the papers were changed every two days to minimize PEG_{6000} buildup (Rehman et al., 1996). To prevent moisture loss, the rolled paper with seeds was placed in sealed, clear plastic bags. For 21 days, seeds were allowed to germinate at 20 ± 1ºC (Perisse et al., 2002) in the dark. The radicles were deemed to have germinated when they reached a length of approximately 2 mm. For ten days, the germination percentage was tracked every 24 hours (Şehirali and Yorgancılar 2011).

The equation Germination percentage (GP) was used to calculate the percentage of seeds that germinated after being subjected to drought stress (Al-Enezi et al., 2012).

$$GP = (NGS/TNS) \times 100 \qquad (1)$$

NGS: Number of germinating seeds
TNS: Total number of seeds

Mean germination time (MGT) was calculated following Ellis and Roberts (1980) to assess the rate of germination.

$$MGT = \Sigma Dn / \Sigma D \qquad (2)$$

Where n is the number of the seeds newly germinated on day D, and D is the number of days from the beginning.

The speed of germination serves as an index for the germination rate (GRI) determined using the following equation (Maguire 1962):

$$GRI = \Sigma\ NGS / \Sigma\ ND \qquad (3)$$

NGS : Number of Germinated Seeds
ND : Number of Days

Seedlings with stunted primary roots and short, thick, spiral-shaped hypocotyls were deemed to have aberrant germination. Initial seedling growth parameters (shoot and root length, shoot and root fresh weights, shoot and root dry weights, shoot and root dry matter, shoot and root water content, and seedling vigor index) were measured after the 21st day. Samples were dried in an oven at 70°C for 48 hours before dry weights were calculated (Beyaz et al., 2011).

$$SVI = (ARK+AHL) \times (GP) \qquad (4)$$

SVI : Seedling vigor index
ARL : Average root length
AHL : Average hypocotyl length
GP : Germination percentage
(Abdul-Baki and Anderson, 1973)

Water content (WC) and dry matter (DM) were calculated formulas;

$$WC = (FW-DW)/FW \times 100 \qquad (5)$$

(Zheng et al., 2008)

$$DM = (DW/FW) \times 100 \qquad (6)$$

(Bres et al., 2022), respectively.

FW : Fresh weight
DW : Dry weight

Statistical Data Analysis

The design completely randomized design with four replications. All recorded data pertaining to seed germination and seedling growth performance were statistically evaluated using IBM SPSS version 22.0 software (Bertsouklis et al. 2022). An analysis of variance (ANOVA) and Duncan's multiple range test were used to investigate potential treatment changes ($p \leq 0.05$). To ensure the homogeneity of the variance, the data of parameters that were calculated as percentages were arcsine transformed before the statistical analysis (Snedecor & Cochran, 1967). The standard error (SE) was computed for each treatment.

Results and Discussion

Effects of Drought Stress on Germination

The crucial stage of a plant's life cycle is germination, which is influenced by several variables, particularly hormones, light, temperature, and moisture availability. The biggest limiting factor for the growth of plants and the germination of seeds, however, is a lack of water.

Drought has a negative impact on the metabolic processes that affect seed germination and ultimately delay the establishment of seedlings. According to several studies, PEG6000 is frequently used to imitate the effects of drought, particularly on seed germination (Ahmed et al., 2022). In this study, the germination of *Lupinus albus* L. in different PEG$_{6000}$ percentage treatments was tested under laboratory conditions. The evolution of the early seed germination parameters according to different percentage treatments of PEG$_{6000}$ is shown in Table 1. The statistical data showed that the effect of increasing percentages of PEG$_{6000}$ on germination percentage (GP), mean germination time (MGT), and germination rate index (GRI) for L. albus seeds was significant ($p \leq 0.01$). Due to increasing percentages of PEG$_{6000}$ in treatments, GP and GRI values decreased while MGT values increased. At 12% PEG$_{6000}$, GP started to decrease by 13.4%. This decrease was sharper (46.7%) in the 16% PEG$_{6000}$concentration. A high germination index is beneficial as it predicts seed strength (Kayacetin, 2022b). Considering the results, the maximum GRI (6.47%) was obtained from the control group, while the lowest germination index (1.20%) was obtained with 16% PEG$_{6000}$ treatment. When the highest drought level (16% PEG$_{6000}$) was compared with the control group, it was determined that MGT increased by 28.09%, while GRI decreased by 81.45%. Overall, when these three germination-parameter data are considered, it was observed that the germination of *Lupinus albus* L. seeds was adversely affected after 12% PEG$_{6000}$, and this adverse effect was even more severe at 16% PEG$_{6000}$. The decline in germination due to the effect of high PEG$_{6000}$concentrations (-0.6 and -0.8 MPa) is in agreement with the previous studies of Perisse et al. (2002) on the L. albus local cultivar “Prima” in Argentina.

The present results agree with those reported by Kaya et al. (2006), who observed that an increase in drought conditions, such as exposure to -0.6 MPa PEG$_{6000}$, induces a reduction in germination parameters of sunflower. In addition, Kayaçetin (2022a) stated that PEG$_{6000}$ treatments (-0.2 and -0.4 MPa) inhibited seed germination in the safflower genotypes.

Table 1. The impact of different percentages of PEG$_{6000}$ on seed germination and growth traits of 21-day-old seedlings.

T#	GP	MGT	GRI	SL	RL
	---%---	---day---	---%---	----------cm----------	
0	100.0±0.00^{a}	5.98±0.04^{b}	6.47±0.72^{a}	8.38±2.20^{a}	6.16±0.76^{a}
4	100.0±0.00^{a}	6.37±0.58^{b}	5.34±1.97^{a}	4.78±1.71^{b}	5.53±0.92^{ab}
8	100.0±0.00^{a}	6.42±0.21^{b}	4.79±1.21^{a}	4.13±0.11^{ab}	5.60±0.60^{ab}
12	86.6±23.09^{a}	7.55±0.05^{a}	2.07±0.37^{b}	1.96±1.34^{bc}	4.55±0.22^{bc}
16	53.3±11.54^{b}	7.66±0.00^{a}	1.20±0.00^{b}	1.11±0.19^{c}	3.94±0.62^{c}
Mean	88.0	6.80	3.97	4.07	5.15
Summary of one-way-ANOVA					
PEG$_{6000}$	**	**	**	**	**
T#	R/S	SFW	RFW	SDW	RDW
	---%---	------------------------mg/plant------------------------			
0	0.71±0.14^{c}	1.89±0.23^{a}	0.22±0.04^{abc}	0.235±0.04^{ab}	0.018±0.001^{b}
4	1.14±0.12^{c}	1.39±0.22^{b}	0.34±0.03^{a}	0.194±0.00^{b}	0.039±0.012^{a}
8	1.35±0.11^{bc}	1.37±0.04^{b}	0.30±0.06^{b}	0.262±0.05^{ab}	0.041±0.012^{a}
12	3.24±2.26^{ab}	1.02±0.12^{c}	0.18±0.07^{c}	0.257±0.03^{ab}	0.033±0.000^{ab}
16	3.55±1.08^{a}	0.82±0.10^{c}	0.12±0.02^{c}	0.299±0.12^{a}	0.021±0.003^{b}
Mean	2.00	1.30	0.23	0.250	0.030
Summary of one-way-ANOVA					
PEG$_{6000}$	**	**	*	*	**

T: Treatments# (%); *Significant at $p \leq 0.05$, **significant at $p \leq 0.01$. Different letters in the same column signify substantial changes at the 0.05 level. #: 4% (-0.03MPa), 8% (-0.1 MPa), 12% (-0.2 MPa), and 16% (-0.3 MPa). Germination percentage (GP), mean germination time (MGT), germination rate index (GRI), shoot length (SL), root length (RL), root to shoot ratio (R/S), shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), and root dry weight (RDW)

Table 2. The impact of different percentages of PEG$_{6000}$ on growth traits of 21-day-old seedlings.

T#	SDM	RDM	R/S DM	SWC	RWC	SVI
	---------------------------------------%--------------------------------------					
0	12.53±2.74b	8.21±0.90b	0.66±0.03c	87.46±2.74a	91.78±0.90a	1455±449a
4	14.23±2.72b	11.45±0.99ab	0.82±0.03a	85.76±2.72a	88.54±0.99ab	1031±394a
8	19.06±3.48b	13.44±2.35ab	0.71±0.07bc	80.93±3.48a	86.55±2.35ab	973±70a
12	25.41±5.39ab	21.48±11.42a	0.81±0.03ab	74.58±5.39b	78.51±11.42b	651±77ab
16	37.79±19.15a	17.59±3.68a	0.53±0.08d	62.20±19.15b	82.40±3.68b	441±146b
Mean	21.80	14.43	0.70	78.19	85.56	910
Summary of one-way-ANOVA						
PEG$_{6000}$	*	*	**	*	*	**

T: Treatments# (%); *Significant at $p \leq 0.05$, **significant at $p \leq 0.01$. Different letters in the same column signify substantial changes at the 0.05 level. #: 4% (-0.03MPa), 8% (-0.1 MPa), 12% (-0.2 MPa), and 16% (-0.3 MPa). Shoot dry matter (SDM), root dry matter (RDM), root to shoot dry matter ratio (R/S DM), shoot water content (SWC), root water content (RWC), and seedling vigor index (SVI).

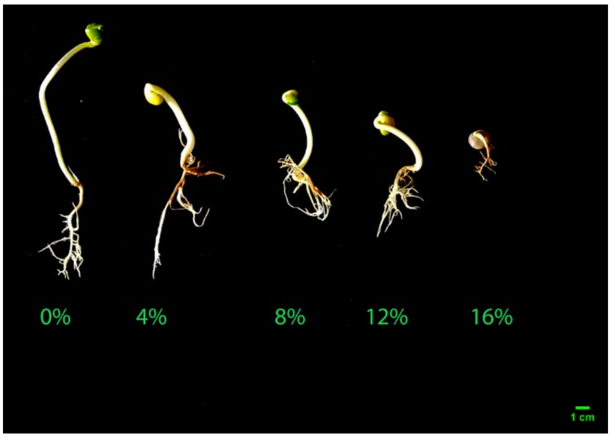

Figure 1. The morphology of 21-day-old seedlings is observed at different percentages of PEG_{6000} treatments.

Pratap and Sharma (2010), Bagheri and Yeganeh (2011), and Baghizadeh and Hajmohammadrezaei (2011) also showed similar restriction of seed germination.

This could be an adaptation mechanism of seeds to limit germination in stressful environments and ensure adequate seedling establishment. It was stated that a decrease in the water potential gradient between seeds and their surrounding media could harm seed germination and subsequent metabolic events in seedling growth and development (Rohamare et al., 2014). Under drought conditions, there was a significant reduction in germination percentage and germination indices, which might be attributed to reduced metabolic processes, such as the synthesis of hydrolytic enzymes and the hydrolysis of dietary material and a delay in both cell division and elongation (Rogan and Simon 1975; Ahmed et al. 2022).

Effects of Drought Stress on Growth Traits

Water scarcity leads to a shortage in the plant tissues very quickly. Desiccation also has an impact on a crop's habitus, development, and metabolism. Dehydration causes substantial physiological changes that delay or even stop growth and jeopardize the stability of crop yield. The impact of drought on crop yield may be particularly significant during specific stages of crop development (Dietz et al., 2021). Initial seedling growth is crucial for plant establishment, and plants are susceptible to drought stress at this period (Jamil et al., 2019). In this study, initial growth parameters of 21-day-old seedlings (Figure 1) were tested under different levels of drought stress, using PEG_{6000}. SL, RL, R/S, SFW, RFW, SDW, RDW, SDM, RDM, R/S DM, SWC, RWC, and SVI are shown in Table 1 and Table 2. All growth parameters examined were found to be statistically significant. SL was reduced by 42.96%, 50.71%, 76.61%, and 86.75% under 4%, 8%, 12%, and 16% PEG_{6000} treatments, respectively. Compared to the control, RL was reduced by 10.22% with 4%, 9.09% with 8%, 26.13% with 12%, and 36.03% with 16% PEG_{6000} treatments.

The R/S was increased by 60.56% under 4% PEG treatment, 90.14% under 8% PEG, 356.34% under 12% PEG, and 400% under 16% PEG, in comparison with the control group. The results of this research showed that as the drought stress level increased, SL and RL decreased, while R/S increased significantly. Our results are consistent with those obtained by Kayaçetin (2022a), who indicated that drought stress imposed with different levels of PEG_{6000}, (-0.2 and -0.4 MPa) decreased SL and RL in sunflower genotypes, while R/S increased. Similarly, Robin et al. (2015) reported that PEG_{6000}-treated hydroponic culture significantly decreased the root length of wheat varieties. In addition, Li et al. (2011) stated that the shoot length of pyrethrum (*Tanacetum cinerariifolium*) was significantly affected at osmotic potentials in PEG solutions less than -0.3 MPa. The root-to-shoot ratio is an important metric for understanding how plants distribute biomass to adapt to stress conditions (Taraves et al., 2021). However, a healthy root system not only helps the plant acquire water, which is a key drought adaptation technique, but also helps the plant's above-ground portions establish (Keresa et al., 2008). Root length at the seedling stage provides an accurate prediction of root growth in the field (Ali et al. 2011a,b; Rajendran et al. 2011). According to our results, in terms of elongation, shoots were more affected than roots by increasing drought levels. Drought stress decreased shoot growth more than root growth and, in some situations, enhanced root growth (Salih et al. 1999; Younis et al. 2000; Okçu et al. 2005; Bibi et al. 2010). Shoot length may be reduced due to reduced water absorption and a decrease in external osmotic potential caused by PEG (Kaydan and Yagmur, 2008). However, considering the PEG_{6000} treatment concentrations, the adverse effect on SL and RL was observed to sharply begin at 12%. Reduced seedling growth is caused by restricted cell division and enlargement due to drought stress (Bibi et al., 2012). The reduced root and shoot length might be attributed to decreased cell division and elongation, which resulted in tuberization and lignification, and subsequently inhibited the plant growth process in the stressed environment (Ahmed et al., 2022).

Under drought stress, SFW decreased and RDW increased, while RFW and SDW showed variability (Table 1). It was observed that RFW increased under 8% PEG_{6000} treatment, compared to the control group, and decreased with 12% and 16% PEG_{6000}. However, it was recorded that RDW increased with 8% PEG_{6000} treatment, compared to the control group, but then decreased with 12% and 16% PEG_{6000} treatments while remaining higher than the control group. In general, considering these results, the shoots of initial growth stage seedlings of *L. albus* were adversely affected after 4% PEG_{6000} treatment (-0.03 MPa -low drought-), while the roots began to be adversely affected after 12% PEG_{6000} treatment (-0.2 MPa -moderate drought-). These results are also supported by the findings of Kaya et al. (2006), who indicated that seedling fresh weight significantly decreased as the osmotic potential of PEG_{6000} solutions decreased, resulting in shorter shoot and root lengths. Similarly, Toscano et al. (2017), Jamil et al. (2019), and Kayaçetin (2022a) observed a decrease in seedling fresh weights of ornamental sunflowers, rapeseed, and safflower genotypes with increasing PEG_{6000} concentrations or decreasing water potential. Contrary to our expectations, seedling dry weights decreased. Bibi et al. (2012) reported that morphological characters such as SFW, RFW, and RDW at the seedling stage are reduced by water stress in sorghum. In addition, Ahmed et al. (2022) stated that increased PEG_{6000} concentrations (10% and 20%) resulted in decreased SFW and RDW in sesame. Robin et al. (2021) reported that, in support of our results, that root dry weight increased in wheat genotypes, which they assumed to be drought tolerant with increasing PEG_{6000} concentrations. The ability of shoots to absorb assimilates as drought stress intensifies may contribute to plant survival and seed filling under stress via retranslocation (Annicchiarico et al., 2018). Therefore, contrary to the information in the literature, the increase in drought severity and shoot dry weight (SDW) detected in our research may indicate that L. albus is engaging its adaptation mechanism against drought. Our results show that shoot growth is more negatively affected than the root growth of *L. albus* under drought stress. Supporting our results, Perisse et al. (2002) reported that the shoot-root dry weight was reduced with decreasing osmotic potentials (0.0, -0.4, -0.6, and -0.8 MPa) in *L. albus* cultivar "Prima," and root growth was dominant as well. In addition, Hessini et al. (2009) found that the shoot-to-root ratio, whether based on fresh or dry weight, diminishes due to water stress, suggesting that shoot growth is more susceptible to this stress than root growth in Spartina alterniflora. Moreover, a similar statement was made by Ashraf and Foolad (2007).

PEG_{6000} solution caused a growth reduction in the SL and RL of *L. albus* seedlings. However, the SDM, RDM, and root to shoot dry matter ratio, (R/S DM) increased with rising drought stress (Table 2). Compared with the control plants, all the PEG_{6000} treatments significantly increased SDM by 13.56%, 52.11%, 102.79%, and 201.59%, and RDM by 39.46%, 63.70%, 161.63%, and 114.25%. However, R/S DM increased by 24.24%, 7.57%, and 22.72% under 4%, 8%, and 12% PEG_{6000} treatments, respectively, while it decreased by 19.69% under 16% PEG_{6000}. Considering R/S DM, it was observed that there was more dry matter accumulation in the root until 12% PEG_{6000} treatment, however, the dry matter ratio decreased with 16% PEG_{6000} (-0.3 MPa). Therefore, according to these results, there is more accumulation in the root under drought stress for L. albus, and the threshold value of this accumulation is 12% PEG_{6000} (-0.2 MPa). Similarly, Atak et al. (2006) reported that increased osmotic stress (-0.45 MPa) caused a decrease in SL and RL in triticale seedlings and an increase in SDM.

SWC and RWC decreased with rising osmotic stress (Table 2). Compared with the control plants, drought stress decreased SWC by 1.94%, 7.46%, 14.72%, and 28.88%, respectively, and RWC by 3.53%, 5.69%, 14.45%, and 10.22%, respectively, under 4%, 8%, 12%, and 16% PEG_{6000} treatments. Considering these cumulative reduction values in treatments, it is observed that water loss in the shoot is greater than in other parts of the plant. A decrease in water contents of shoots could result from a decrease in water flow from the roots to the respective shoots (Kayaçetin, 2022a). The decrease in the amount of water in roots and shoots can be explained by the decrease in root length, which is due to low water potential, resulting in reduced water uptake capacity of the root. Similarly, Bajji et al. (2000) reported that water contents of the roots and shoots of wheat cultivar seedlings were reduced by water stress.

Increasing concentrations of PEG_{6000} solutions induced a marked reduction in SVI of *L. albus* (Table 2). Compared with the control application, drought stress decreased SVI by 29.14%, 33.12%, 55.25%, and 69.69%, under 4%, 8%, 12%, and 16% PEG_{6000} treatments, respectively. These results showed that SVI decreased drastically at 12% PEG_{6000}. Therefore, in terms of SVI, it is understood that *L. albus* will suffer significant yield loss at 12% PEG_{6000} (-0.2 MPa) and concentrations more negative than -0.2 MPa. The findings of Kayaçetin (2022a) confirmed our results, and as the water potential decreased with increasing PEG_{6000} concentrations, a significant decrease in the seedling vigor index adversely affected the vegetative growth of safflower genotypes. In addition, this result was consistent with the findings of Ahmed et al. (2022), which showed the PEG_{6000} induced osmotic impact decreased the vigor index of sesame seedlings. Furthermore, our findings showed a diminution in the SVI after seedlings' exposure to drought, which is in agreement with reports of Kouighat et al. (2021) in mutant sesame, Spielmeyer et al. (2007) in wheat, and Koskosidis et al. (2020) in chickpeas.

Conclusion

Spring and early summer drought, which coincides with critical reproductive stages, severely limits *L. albus* output in Mediterranean climate zones. Therefore, the response of *L. albus* to drought should be well understood. In this study, germination and initial seedling growth, which are very critical in drought, were examined, as in other abiotic stress factors. As a result of the study, it was determined that the negative effect of drought stress begins at 12% PEG_{6000}, (-0.2 MPa) treatment for germination. However, initial seedling growth was adversely affected after 4% PEG_{6000} (-0.03 MPa), and this adverse effect was exacerbated by higher PEG_{6000} treatments (12% and 16%). Therefore, in light of these data, we conclude that the initial seedling growth period in *L. albus* is more sensitive than the germination period under drought stress. However, when looking at shoot and root growth parameters, it was seen that the responses to drought stress were different, and shoot growth was more negatively affected than root growth. Overall, according to our results, *L. albus* would be classified as a species susceptible to drought, especially in the initial growth stage.

Declarations

Author Contribution Statement

R.B.: Data collection, investigation, conceptualization, methodology and writing the original draft

V.V.U.: Review and editing

Conflict of Interest

The authors declare no conflict of interest.

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