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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Effects of Iso-Osmotic Potential of NaCl and PEG_{6000} Solutions on Germination and Initial Seedling Growth of Sweet White Lupin (*Lupinus albus* L)

İzo-Ozmotik NaCl ve PEG_{6000} Solüsyonlarının Tatlı Beyaz Acı Baklanın (*Lupinus albus* L.) Çimlenme ve İlk Fide Gelişimi Üzerine Etkileri

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Abstract

Sweet white lupin has a growing importance as a staple crop due to its rich protein and mineral content. Investigation of unfavorable environmental conditions at the seedling stage is critical for understanding and overcoming the challenges during germination and initial growth. In this study, the effects of salt and drought stress were investigated upon induction of NaCl and PEG_{6000} osmotic agents at iso-osmotic potential (0, -0.3 and -0.6 MPa) on seed germination and initial seedling growth in sweet white lupin (*Lupinus albus* L.). The research was carried out for 21 days under laboratory conditions according to a completely randomized plot design with 4 replicates. In order to assess the growth upon stress, germination percentage, mean germination time, germination rate index, shoot and root length, root/shoot length, shoot and root fresh weight, shoot and root dry weight, shoot and root dry matter content, root/shoot dry matter content, shoot and root water content and seedling vigor index parameters were measured. The results demonstrated that NaCl and PEG_{6000} solutions applied at the same osmotic potential had statistically significant effects on the measured germination and growth parameters. PEG_{6000} treatments at the same osmotic potential had more adverse effects on germination and initial seedling growth than NaCl treatments. In addition, shoot growth was more adversely affected than root growth in PEG_{6000} and NaCl treatments. The germination was limited in -0.6 MPa PEG_{6000} treatment and no subsequent seedling growth was observed. In this study, we documented that the white lupine's tolerance to drought during germination and initial seedling growth periods was lower than salinity at the same iso-osmotic potential and saline and arid soils showing an osmotic water potential of -0.6 MPa (12.7 dS m^{-1} EC and 22% PEG_{6000}) are inhibitory for lupin germination and growth. This study lays the ground for further physiological and molecular studies on the effects of salt and osmotic stress on white lupins.

Keywords: *Lupinus albus* L., Iso-osmotic potential, NaCl, PEG_{6000}, Germination, Initial seedling growth

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Öz

Tatlı beyaz acı bakla, zengin protein ve mineral içeriği nedeniyle temel bir ürün olarak giderek artan bir öneme sahiptir. Fide aşamasında elverişsiz çevre koşullarının araştırılması, çimlenme ve erken büyüme sırasındaki zorlukların anlaşılması ve üstesinden gelinmesi için kritik öneme sahiptir. Bu çalışmada, tatlı beyaz acı baklada (*Lupinus albus* L.) tohum çimlenmesi ve ilk fide büyümesi üzerine NaCl ve PEG_{6000} ozmotik ajanlarının izo-ozmotik potansiyelde (0, -0,3 ve -0,6 MPa) indüksiyonu ile oluşturulan tuz ve kuraklık stresinin etkileri araştırılmıştır. Araştırma, tesadüfi parseller deneme desenine göre 4 tekerrür olacak şekilde laboratuvar koşullarında 21 gün boyunca yürütülmüştür. Stres altındaki büyümeyi değerlendirmek amacıyla çimlenme yüzdesi, ortalama çimlenme süresi, çimlenme indeksi, sürgün ve kök uzunluğu, kök/sürgün oranı, sürgün ve kök taze ağırlığı, sürgün ve kök kuru ağırlığı, sürgün ve kök kuru madde içeriği, kök/sürgün kuru madde içeriği, sürgün ve kök su içeriği ve fide canlılık indeksi parametreleri ölçülmüştür. Sonuçlar, aynı ozmotik potansiyelde uygulanan NaCl ve PEG_{6000} çözeltilerinin ölçülen çimlenme ve büyüme parametreleri üzerinde istatistiksel olarak anlamlı etkilere sahip olduğunu göstermiştir. Aynı ozmotik potansiyelde PEG_{6000} uygulamaları, NaCl uygulamalarına kıyasla çimlenme ve ilk fide büyümesi üzerinde daha olumsuz etkilere sahip olmuştur. Ayrıca, PEG_{6000} ve NaCl uygulamalarında sürgün büyümesi kök büyümesine kıyasla daha olumsuz etkilenmiştir. Çimlenme -0.6 MPa PEG_{6000} uygulamasında sınırlı kalmış ve sonuç olarak fide büyümesi gözlenmemiştir. Bu çalışmada, tatlı beyaz acı baklanın çimlenme ve ilk fide büyüme dönemlerinde kuraklığa karşı toleransının, aynı izo-ozmotik potansiyeldeki tuzluluktan daha düşük olduğunu ve -0.6 MPa (12,7 dS/m EC ve %22 PEG_{6000}) ozmotik su potansiyeli gösteren tuzlu ve kurak toprakların acı bakla çimlenmesi ve büyümesi için engelleyici olduğunu belgelenmiştir. Bu çalışma, tuz ve ozmotik stresin beyaz acı bakla üzerindeki etkileri konusunda daha ileri fizyolojik ve moleküler çalışmalar yapılmasına zemin hazırlamaktadır.

Anahtar Kelimeler: *Lupinus albus L.,* İzo-ozmotik potansiyel, NaCl, PEG_{6000}, Çimlenme, İlk fide büyümesi

1. Introduction

Drought and salinity are two of the the most critical agricultural environmental challenges worldwide and the consequences are likely to worsen in the course of current climate change (Giordano et al., 2021). The negative impacts of water scarcity and high salinity on plants can be seen at the whole-plant level as plant mortality and/or decreased yield (Zheng et al., 2010). High salinity decreases plants' water uptake capacity, leading to a rapid decline in growth rate and triggering a set of metabolic changes similar to those induced by water stress (Munns, 2002). Accumulation of soluble salts in soils increases the osmotic pressure of the soil solution, potentially impeding water absorption by seeds or plant roots. Salt damage to plants is caused by reduced water availability, toxicity of certain ions, and nutritional imbalance resulting from these ions (Murillo-Amador et al., 2002).

Salt stress arises from the accumulation of elevated concentrations of Cl^- or Na^+ ions in the soil, resulting in ion toxicity in plants. While plant reactions to salinity differ among species, excessive absorption of Na^+ typically results in nutritional imbalance. The Na^+ ion reaches lethal concentrations before the Cl^- ion. Elevated concentrations of Na^+ ions adversely affect photosynthetic activity and may exacerbate outcomes in salt-sensitive plants. Although Na^+ ions may have detrimental effects on certain plants, Cl^- ions may be even more harmful in species such as soybeans and citrus. Chloride ions (Cl^-) can impair photosynthetic activity and may induce ion toxicity in plants, similar to sodium ions (Na^+). In reaction to ion toxicity, the plant-produced hormone abscisic acid (ABA) is crucial. The synthesis rate escalates during stress to avert disturbances in cellular growth and development systems (Yildiz et al., 2020). Certain ions must be present within plant cells for growth. Despite the presence of ions such as nitrogen, potassium, and calcium in the soil, their entry into plant cells is impeded by competition with other ions at elevated quantities. The presence of excessive concentrations of salt in soil hinders the absorption of ions essential for plant development. Plants initiate several reactions to mitigate this adverse effect and maintain low ion concentrations. Distributing ions for plant addition rather than allowing cellular absorption simultaneously is regarded as a crucial action for plant growth and development. The cell membrane is crucial for sustaining low ion concentrations during transit to plants. Proteins, channel proteins, and symporters facilitate the transport of ions into the plant. Antiporters are utilized in transport mechanisms. These transporters are situated in vacuolar membranes. V-ATPases are recognized as essential channels for plant survival under saline stress. The excessive concentration of Na^+ ions in the soil prompts their movement into the cytoplasm, where they are transported to vacuoles via Na^+/H^+ antiporters. In plant cell metabolism, another function is the maintenance of cytoplasmic K^+ homeostasis. In the presence of salinity, the concentration of K^+ experiences a significant reduction. K^+ ions, which can be transported into cells via K^+ transporters and membrane channels, exhibit low concentrations during salinity stress. An essential element in cell recruitment exists. When extracellular K^+ concentration is low, K^+ transporters facilitate high-affinity K^+ absorption processes, which are activated when extracellular K^+ concentration is elevated. Consequently, the concentration of Na^+ ions escalates in saline conditions, leading to Na^+ competing with K^+ and diminishing K^+ uptake into the cell. Increased K^+ retention in the roots of plants such as wheat, maize, and beans has been identified as a method employed by these plants to endure salt stress. The accumulation of K^+ ions in cells rises under salinity stress (Yildiz et al., 2020).

However, water deficit causes other damaging effects on plant growth including epinasty, stomatal closure, and decreased photosynthesis (Lei et al., 2021). In addition, these environmental factors cause osmotic imbalance in plants by lowering the water potential of their environment. Osmotic stress causes cell elongation to be inhibited, stomata to close, photosynthetic activity to be reduced, abnormalities in water and ion intake, assimilate translocation, and changes in numerous metabolic activities (Çakmakçı and Dallar, 2019; Darko et al., 2019). PEG is primarily utilized to obtain data from plants concerning drought stress. PEG is unable to permeate the plant cell wall and possesses a higher molecular weight than the other osmotic agents employed, particularly PEG_{6000}. Consequently, it is often employed in germination and drought studies to regulate osmotic potential (Beyaz, 2023). Drought and salinity are the key environmental conditions that limit crop establishment success, and seed germination is the first important and most sensitive stage in the life cycle of plants (Benlioglu and Ozkan, 2020; Zuffo et al., 2020).

Lupins are a significant economic crop, and their seeds are a key part of animal feed. Especially sweet white lupins gained further economic importance due to their increasing role in human nutrition. Through their symbiotic relationship with N_2-fixing bacteria, they also play a critical part in maintaining long-term soil fertility. Lupins are

normally grown on well-drained acidic to neutral soils and are native to the Mediterranean region. Among the main obstacles to lupin production are drought and soil-related challenges including salt and nitrogen deficit (Yu and Rengel, 1999). There are limited reports about the responses of *L. albus* to salinity (Yu and Rengel, 1999; Fernades et al., 2004; Slabu et al., 2010; Hussien, 2022) and drought (Yu and Rengel, 1999; Perisse et al., 2002; Pinheriro et al., 2004; Slabu et al., 2010; Annicchiarico et al., 2018; Pecetti et al., 2023). However, the effects of of salt and drought stress at the iso-osmotic potantial concentrations on germination and initial seedling growth of *L. albus* remain elusive. Therefore, the aim of this study is to determine and compare the responses of *L. albus* L. under iso-osmotic drought and salt stress conditions.

2. Materials and Methods

2.1. Seed Materials

Lupinus albus L. seeds (2023) were obtained from RLP AgroScience GmbH (Germany) were used as plant material in the study. This study was carried out at Kırşehir Ahi Evran University (Türkiye), Faculty of Agriculture, Department of Agricultural Biotechnology.

2.2. Preparation of iso-osmotic potential test solution with NaCl and PEG_{6000}

Test solutions were prepared according to Kaya et al. (2006) and Li et al. (2011) using distilled water. Accordingly, briefly, 3.5 and 7.1 g of NaCl (Coons et al., 1990) were added to 1 liter of distilled water to prepare -0.3 MPa (=60 mM) and -0.6 MPa (=121 mM) NaCl osmotic potential solution, respectively. To prepare -0.3 MPa and- 0.6 MPa PEG osmotic potential solution, 151.4 and 223.6 g of PEG (Michel and Kaufmann, 1973) were added to 1 liter of distilled water, respectively. The electrical conductivity (EC) values of NaCl solutions were 6.5 and 12.7 dS m^{-1} as reported by Kaya et al. in 2006. Distilled water (0 MPa) was used as control.

2.3. Germination tests and morphological observations

With 10 ml of each test solution, three duplicates of 10 seeds were planted between three rolls of filter paper. Before planting, seeds were given a fungicide treatment (Thiram 80%), and papers were changed every two days to minimize the buildup of NaCl and PEG_{6000} concentrations (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 degrees (Perisse et al., 2002) in the dark in incubator (Memmert-In110). The radicles were deemed to have germinated when they reached a length of ~ 2 mm. For ten days, the germination percentage was tracked every 24 hours (Şehirali and Yorgancılar, 2011).

Equation -germination percentage $(GP) = (\text{Number of germinating seeds}/\text{ Total number of seeds}) \times 100$ (Eq. 1) (Al-Enezi et al., 2012)-was used to calculate the proportion of seeds that germinated after being subjected to drought stress.

To assess the rate of germination, the mean germination time (MGT) was determined (Ellis and Roberts, 1980).

$$MGT = \sum Dn/\sum D \quad \text{(Eq. 2)}$$

where n is the number of freshly germinated seeds on day D, and D is the number of days since the start of the experiment. The germination rate index (speed of germination index) was calculated using the formula according to Maguire (1962) (Eq 3.)

$$GRI = \Sigma \text{ No of Germinated Seeds}/\ \Sigma \text{ No of Days} \quad \text{(Eq 3.).}$$

Seedlings with stunted primary roots and short, thick, spiral-shaped hypocotyls were deemed to have aberrant germination. Initial seedling growth paramaters (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 21th day . Samples were dried in an oven at 70°C for 48 hours before dry weights were calculated (Beyaz et al., 2011). The following parameters were used to calculate the growth parameters.

$$\text{Water content (WC)} = (\text{fresh weight} - \text{dry weight})/\text{fresh weight} \times 100 \text{ (Zheng et al., 2008)} \quad \text{(Eq. 4)}$$

$$\text{Dry matter (DM)} = (\text{dry weight}/\text{fresh weight}) \times 100 \text{ (Bres et al., 2022)} \quad \text{(Eq. 5)}$$

$$\text{Seedling vigor index (SVI)} = (\text{average root length} + \text{average hypocotyl length}) \text{ x germination percentage (GP)}$$

(Abdul-Baki and Anderson, 1973) (Eq. 6)

2.4. Data Analysis

The experiment was set up a completely randomized plot experimental desing, with 4 repetitions per treatment and 20 seeds in each repitation. The percentage data underwent Arcsine transformation before being analyzed using One-way analysis of variance in the SPSS statistical tool (Version 22) (Snedecor and Cochran, 1967). The Duncan's Multiple Range Test (DMRT) was utilized to compare the mean differences at a significance level of $P \leq 0.05$.

3. Results and Discussion

In this study, the effects of different levels of salt and drought stress using NaCl and PEG_{6000} osmotic agents at the same osmotic potential on seed germination and initial seedling growth in *L. albus* were investigated.

3.1. Effects of NaCl and PEG_{6000} solutions at the same osmotic potential on germination

The seeds were germinated in different NaCl and PEG_{6000} solutions. A stronger inhibition of germination and growth inhibition was readily observable in higher NaCl and PEG_{6000} solutions (*Figure 1*). Germination percentage (GP -except NaCl treatments-), mean germination time (MGT) and germination rate index (GRI) were significantly ($P \leq 0.01$) affected in both treatments. Depending on decreasing osmotic potentials of NaCl solutions, although GP did not change, the MGT increased and GRI decreased. When the control group (0 MPa) was compared with the highest NaCl solution (-0.6 MPa), there was an 8.76% increase in MGT and a 37.64% decrease in GRI (*Table 1*). However, with the decreasing osmotic potential of PEG_{6000} solutions, GP and MGT increased, but GRI decreased. Compared to the control, GP and GRI reduced by 66.67% and 91.92% under -0.6 MPa PEG_{6000} treatment. On the other hand, MGT increased by 39.45% under -0.6 PEG_{6000} solution (*Table 2*). Salinity and drought affect plants with different mechanisms and plants respond differently to these stress factors. The effects of salt and drought stress on germination in plants occur in two ways. Salt stress both creates stress by limiting the osmotic potential of water and causes ion toxicity (Demir and Mavi, 2008). Drought stress, on the other hand, has an adverse effect by limiting the osmotic potential of water. In general, when the effects of NaCl and PEG_{6000} applied at the same osmotic potential on germination in *L. albus* were examined in this study, it was seen that PEG_{6000} treatments representing drought had more adverse effects than NaCl treatments representing salinity. Similar to these results, Li et al. (2011) reported that at the same osmolarity the inhibition of germination of pyrethrum (*Tanacetum cinerariifolium*) by PEG was stronger than by NaCl. Kaya et al. (2006) stated that inhibition of germination of sunflower (*Helianthus annuus* L.) at the same water potential of NaCl and PEG resulted from osmotic effect rather than ion toxicity. Murillo-Amador et al. (2002) revealed that salt (NaCl) stress had a lower effect on cowpea (*Vigna unguiculata* L. Walp.) seed germination in terms of the germination rate, the emergence rate, and the final germination and emergence percentage than water stress induced by PEG_{8000} at the same osmotic potentials. Moreover, Yagmur and Kaydan (2008) reported that the adverse effect of PEG_{6000} was higher than NaCl on germination percentage of triticale (*Triticosecale Witm*., cv. Presto) at the same osmolarity. Other researchers also reported that PEG had a more adverse effect on germination than NaCl at the same osmolarity (Khajeh-Hosseini et al., 2003; Demir and Mavi, 2008; Moosavi et al., 2009; Tavares et al., 2021). NaCl and PEG at the same osmotic potentials reduced water absorption in seeds, but PEG showed a more pronounced effect in this reduction, indicating that ion uptake and osmotic adjustment occured in seeds treated with NaCl solutions (Murillo-Amador et al., 2002).

3.2. Effects of NaCl and PEG_{6000} solutions at the same osmotic potential on seedling growth

In 21 day old seedlings, we observed that both NaCl and PEG_{6000} applied at different osmotic potentials affected the shoot and root growth parameters significantly differently (except root to shoot, root dry matter, and root water content) (*Table 1,2,3,4,5,6*). Compared to the control group, there was a decrease in shoot and root length due to the NaCl and PEG_{6000} solutions at reduced osmotic potential. Compared to the control, there was a decrease of 40.09% and 44.58% for shoot and root in the NaCl treatment at -0.3 MPa osmotic potential, while there was a decrease of 89.08% and 44.45% for shoot and root in the PEG_{6000} treatment at the same osmotic potential. No measurement could be made in the PEG_{6000} treatment at -0.6 MPa osmotic potential for shoot and root. Therefore, it was observed that PEG_{6000}, i.e. drought stress, had more adverse effects than NaCl treatments, i.e. salt stress, in terms of root and shoot growth. In several plant organs, an increase in the expression, activity, and/or concentration of aquaporins, commonly referred to as aquaporin water channels, has been noted in response to NaCl, but not to PEG, which is positively correlated with the reduced sensitivity of seeds to salt stress (Tavares et al., 2021).

However, when the data from the NaCl solution at the lowest osmotic potential applied in the study (-0.6 MPa) were considered with the control group, there was a decrease of 51.83% and 44.08% in shoots and roots, respectively, and again, when the data from the PEG_{6000} solution at the lowest osmotic potential applied in the study (-0.3 MPa) were considered with the control group, there was a decrease of 89.08% and 44.45% in shoots and roots, respectively. Therefore, it can be speculated that shoots were affected more adversely than roots in both NaCl and PEG_{6000} treatments. Similarly, Perisse et al. (2002) indicated that shoot growth in *Lupinus albus* cultivar "Prima" was much more affected than root growth under different water potentials (-0.4, -0.6, and -0.8 MPa) of PEG_{6000}. A possible explanation was offered by Munss (2002) who indicated, that the initial decrease in shoot growth was most likely due to hormonal cues produced by the roots. Roots might appear to be the most vulnerable portion of the plant since they are directly exposed to salt or drying soil, but they are surprisingly resistant. The root-to-shoot ratio is an important parameter for understanding how plants distribute biomass to adapt to stress conditions. Plants enhance root growth by making nutrients (including water) available. The root/shoot ratio increases under saline conditions (Tavares et al., 2021). In this study, it was observed that the data in the root to shoot parameter in the NaCl and PEG_{6000} solutions at decreasing osmotic potential supported this situation (*Table 1* and *2*). Tavares et al., (2021) reported that increased R/S for water uptake in cowpea seedling under iso-osmotic stress (PEG_{6000} and NaCl) conditions. Overall, increased salt and drought stress adversely affected both shoots and roots. Similarly, Li et al. (2011) reported that salt and drought (at ≤-0.9 MPa NaCl and ≤-0.6 MPa PEG_{6000} osmotic potentials) stress were suppressed both shoot and root growth in pyrethrum (*Tanacetum cinerariifolium*). In addition, Yu and Rengel (1999) stated that elongation rate of shoot and root, depressed both salt and drought (osmotic potentials of -1.4 and -1.8 MPa) stress in narrow-leafed lupins (*Lupinus angustifolius L.*).

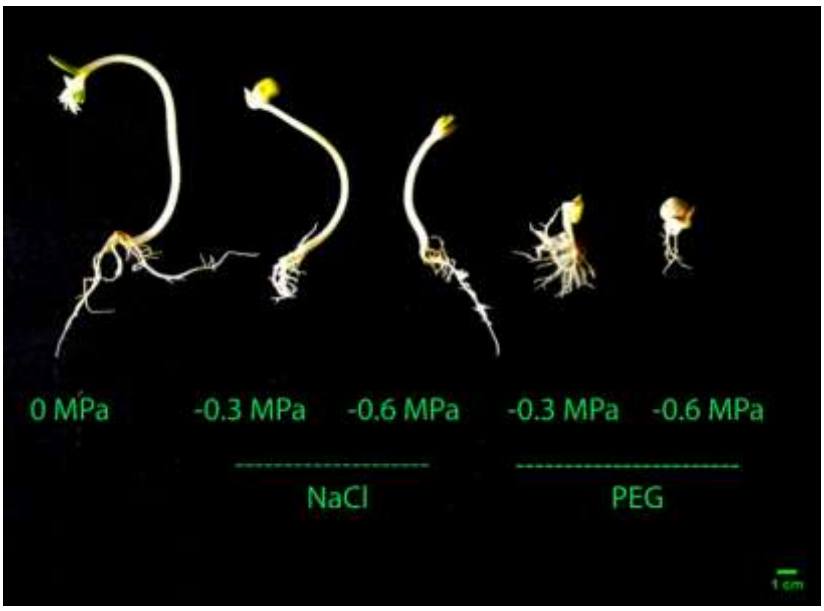

Figure 1. The morphology of Lupinus albus L. seedlings after iso-osmotic potentials of NaCl and PEG_{6000} treatments (after 21 days of germination)

Shoot and root fresh weight, shoot and root dry weight of the *L. albus* seedlings were significantly impacted by the drought stress induced by the PEG_{6000} solutions and by the salt stress induced by the NaCl solutions (*Table 3* and *4*). When compared with the control group, we observed that shoot and root fresh weight decreased in NaCl treatments at decreasing osmotic potential. This decrease was 41.17% and 38.70% for shoot and root in -0.6 MPa NaCl treatment, respectively (*Table 3*). However, shoot and root fresh weight decreased in PEG_{6000} treatments at decreasing osmotic potential, compared with the control group. This decrease was 57.91% and 41.93% for shoot and root in -0.3 MPa PEG_{6000} treatment, respectively (*Table 4*). On the other hand, when compared with the control group, we documented that shoot dry weight increased and root dry weight decreased in NaCl treatments at decreasing osmotic potential (*Table 3*). In -0.6 MPa NaCl treatment, shoot dry weight increased by 0.89% and root dry weight decreased by 22.58%. However, when compared with the control group, both shoot and root dry weight increased in PEG_{6000} treatments at decreasing osmotic potential (*Table 4*). This increase was 10.76% and 12.90% for shoot and root, respectively, in -0.6 MPa PEG_{6000} treatment. Under NaCl treatments at decreasing osmotic

Table 1. Effects of different osmotic potential of NaCl solutions on germination percentange (GP), mean germination time (MGT), germination rate index (GRI), shoot lenght (SL), root lenght (RL), root to shoot (R/S).

Osmotic Potentials (MPa)	GP (%)	MGT (day)	GRI (%)	SL (cm)	RL (cm)	R/S (%)
0	100.00±0.00	4.79±0.08^{b}	6.19±0.28^{a}	10.90±0.41^{a}	7.94±0.60^{a}	0.74±0.18
-0. 3 NaCl	100.00±0.00	5.10±0.03^{a}	4.91±0.19^{b}	6.53±0.57^{b}	4.40±0.10^{b}	0.68±0.06
-0.6 NaCl	100.00±0.00	5.21±0.06^{a}	3.86±0.17^{c}	5.25±0.38^{b}	4.44±0.61^{b}	0.84±0.10
Means	100	5.03	4.98	7.56	5.59	0.75
Summary of ANOVA						
	ns	**	**	**	**	ns

*significant at $P \leq 0.05$, **$P \leq 0.01$, ns:non-significant. Different letters at the same column show significant differences at 0.05 level. ±: Standart Error

Table 2. Effects of different osmotic potential of PEG_{6000} solutions on germination percentange (GP), mean germination time (MGT), germination rate index (GRI), shoot lenght (SL), root lenght (RL), root to shoot (R/S).

Osmotic Potentials (MPa)	GP (%)	MGT (day)	GRI (%)	SL (cm)	RL (cm)	R/S (%)
0	100.00±0.00^{a}	4.79±0.08^{b}	6.19±0.28^{a}	10.90±0.41^{a}	7.94±0.60^{a}	0.74±0.18^{b}
-0.3 PEG	66.66±6.66^{b}	6.52±0.02^{a}	1.07±0.08^{b}	1.19±0.09^{b}	4.41±0.30^{b}	3.71±0.17^{a}
-0.6 PEG!	33.33±3.33^{c}	6.68±0.17^{a}	0.50±0.14^{c}	-	-	-
Means	66.66	5.99	2.58	4.03	4.11	1.48
Summary of ANOVA						
	**	**	**	**	**	**

*significant at $P \leq 0.05$, **$P \leq 0.01$. Different letters at the same column show significant differences at 0.05 level. ±: Standart Error

!: Not enough material could be obtained for parameter measurements.

Table 3. Effects of different osmotic potential of NaCl solutions on shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), root dry weight (RDW), shoot dry matter (SDM), root dry matter (RDM).

Osmotic Potentials (MPa)	SFW (mg/plant)	RFW (mg/plant)	SDW (mg/plant)	RDW (mg/plant)	SDM (%)	RDM (%)
0	2.21±0.29^{a}	0.31±0.02^{a}	0.223±0.006^{b}	0.031±0.002^{a}	10.12±0.52^{b}	9.90±0.81
-0. 3 NaCl	1.39±0.04^{b}	0.14±0.01^{b}	0.265±0.008^{a}	0.024±0.002^{a}	19.09±0.52^{a}	9.15±2.28
-0.6 NaCl	1.30±0.07^{b}	0.19±0.02^{b}	0.225±0.002^{b}	0.013±0.003^{b}	17.25±0.91^{a}	12.26±2.50
Means	1.63	0.21	0.237	0.022	15.48	10.43
Summary of ANOVA						
	*	**	**	**	**	ns

*significant at $P \leq 0.05$, **$P \leq 0.01$, ns: non-significant. Different letters at the same column show significant differences at 0.05 level. ±: Standart Error

Table 4. Effects of different osmotic potential of PEG_{6000} solutions on shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), root dry weight (RDW), shoot dry matter (SDM), root dry matter (RDM).

Osmotic Potentials (MPa)	SFW (mg/plant)	RFW (mg/plant)	SDW (mg/plant)	RDW (mg/plant)	SDM (%)	RDM (%)
0	2.21±0.29^{a}	0.31±0.02^{a}	0.223±0.006^{b}	0.031±0.002^{a}	10.12±0.52^{b}	9.90±0.81^{b}
-0.3 PEG	0.93±0.05^{b}	0.18±0.02^{b}	0.247±0.009^{a}	0.035±0.002^{a}	26.32±1.61^{a}	19.13±1.16^{a}
-0.6 PEG$^{!}$	-	-	-	-	-	-
Means	1.04	0.16	0.15	0.022	12.14	9.67
Summary of ANOVA						
	**	**	**	**	**	**

*significant at $P \leq 0.05$, **$P \leq 0.01$. Different letters at the same column show significant differences at 0.05 level. ±: Standart Error
!: Not enough material could be obtained for parameter measurements.

Table 5. Effects of different osmotic potential of NaCl solutions on shoot dry matter (R/S DM), shoot water content (SWC), root water cantent (RWC), seedling vigor index (SVI).

Osmotic Potentials (MPa)	R/S DM (%)	SWC (%)	RWC (%)	SVI
0	0.98±0.01[a]	89.87±0.52[a]	90.09±0.81[a]	1884±160[a]
-0. 3 NaCl	0.48±0.03[c]	80.90±0.55[b]	90.84±2.28[a]	1093±58[b]
-0.6 NaCl	0.70±0.05[b]	82.74±0.91[b]	87.73±2.50[ab]	969±89[b]
Means	0.72	84.50	89.55	1315
Summary of ANOVA				
	**	**	ns	**

*significant at $P \leq 0.05$, **$P \leq 0.01$. Different letters at the same column show significant differences at 0.05 level. ±: Standart Error

Table 6. Effects of different osmotic potential of PEG_{6000} solutions on shoot dry matter (R/S DM), shoot water content (SWC), root water cantent (RWC), seedling vigor index (SVI).

Osmotic Potentials (MPa)	R/S DM (%)	SWC (%)	RWC (%)	SVI
0	0.98±0.01[a]	89.87±0.52[a]	90.09±0.81[a]	1884±160[a]
-0.3 PEG	0.73±0.01[b]	73.67±1.66[b]	80.86±1.16[b]	378±64[b]
-0.6 PEG[!]	-	-	-	-
Means	0.57	54.51	56.98	754
Summary of ANOVA				
	**	**	**	**

*significant at $P \leq 0.05$, **$P \leq 0.01$. Different letters at the same column show significant differences at 0.05 level. ±: Standart Error

!: Not enough material could be obtained for parameter measurements.

potential, when shoot and root dry matter were considered, there was an increase in both (except -0.3 MPa) (*Table 3*). When the lowest NaCl potential (-0.6 MPa) was compared with the control, the increase was 70.45% and 23.83% for shoot and root dry matter, respectively. However, under PEG_{6000} treatments at decreasing osmotic potential, when shoot and root dry matter were considered, it was observed that there was an increase in both (*Table 4*). When the lowest NaCl potential (-0.3 MPa) was compared with the control, this increase was 160.07% and 93.23% for shoot and root dry matter, respectively. When compared with the control group, it was observed that the root/shoot dry matter ratio decreased due to the decreasing osmotic potential in NaCl treatments (*Table 5*). This decrease was 51.02% in -0.3 MPa NaCl treatment and 28.57% in -0.6 MPa NaCl treatment. In addition, when compared with the control group, it was observed that the root/shoot dry matter ratio decreased due to the decreasing osmotic potential in PEG_{6000} treatments (*Table 6*). This decrease was 25.51% in -0.3 MPa NaCl treatment.

Overall, the results of the study showed that PEG_{6000} treatments had a stronger effect on shoot and root fresh weight, shoot and root dry weight, and shoot and root dry matter than NaCl treatments. NaCl treatments at decreasing osmotic potential had more adverse effects on shoots in terms of fresh weight, and on roots in terms of dry weight and dry matter. However, PEG_{6000} treatments at decreasing osmotic potential had more adverse effects on shoots in terms of fresh and dry weight, and on roots in terms of dry matter. Yu and Rengel (1999) stated that growth parameters such as shoot and root fresh weight restricted under both salt and drought (osmotic potentials of -1.4 and -1.8 MPa) stress in narrow-leafed lupins (*L. angustifolius* L.). Slabu et al. (2010) indicated that excess NaCl in the soil decreases vegetative development by reducing the quantity of accumulated biomass in white lupin cultivars. In addition, Perisse et al. (2002) reported that water potentials of -0.4 and -0.6 MPa PEG_{6000} significantly reduced seedling growth of *L. albus* cultivar "Prima" developed for the central region of Argentina. Moreover, in term of dry matter, similar results were reported by Zheng et al. (2010), who showed that iso-osmotic PEG_{6000} and NaCl treatments (-0.44 and -0.88 MPa) decreased plant dry matter accumulation rate (DMAR) of sunflower (*Helianthus annuus* L.) seedlings. Also, Beyaz et al. (2011) reported that increasing NaCl (5, 10, 20, and 30 dS/m) levels causes increase dry matter of sainfoin seedlings. Zuffo et al. (2020) emphasized that the buildup of shoot dry matter was severely hampered by salt and drought stressors in soybean genotypes, particularly in drought circumstances. Panneerselvam et al. (1998) indicated that NaCl stress decreased dry matter production of *Glycine max* seedlings. The reduction in seedling shoots dry matter due to the solution's osmotic potential is linked to a slowdown in physiological and biochemical processes, as well as challenges in hydrolysis and mobilization of reserves in the seeds caused by water deficiency (Sousa et al., 2018).

Another result of the research findings is that the NaCl solutions [(-0.3 MPa, =60 mM NaCl) and (-0.6 MPa, =121 mM NaCl)] with different osmotic potentials applied in the study have no effect on the germination percentage, which is one of the germination parameters (*Table 1*). On the other hand, the adverse effect on the vegetative growth of *L. albus* seedlings begins immediately at -0.3 MPa NaCl treatment (*Table 1, 3*, and *5*). Similar to our results, Tavares et al. (2021) reported that low concentrations of NaCl (≤75 mM) did not affect germination, but adverse affected growth. They noted that salt accumulation in plant tissues causes multiple physiological, morphological, and biochemical alterations in plant metabolism, resulting in a reduction in cell wall expansion and restriction of photosynthetic CO_2 uptake.

Seedling vigor index was calculated as 1884, 1093, 969 and 378 under control (0 MPa), -0.3 MPa NaCl, -0.6 MPa NaCl and -0.3 MPa PEG_{6000} treatments, respectively (*Table 5* and *6*). Seedling vigor index decreased due to increasing salt and drought stress, this decrease was more intense in drought stress at iso-osmotic potential. Similarly, Ghiyasi et al. (2019) reported that PEG_{6000} caused a decrease in seedling vigor index in black cumin (*Nigella sativa* L.) with its decreasing osmotic potential. Also, Borsai et al. (2018) indicated that NaCl-induced salt stress and PEG_{6000}-induced drought stress at the same osmotic potentials causes decreasing in seedling vigor index of portulaca.

4. Conclusions

In conclusion, the results of the study showed that both germination and initial seedling growth in *L. albus* were adversely affected by PEG_{6000} and NaCl treatments at the same osmotic potential. This adverse effect was greater in PEG_{6000} treatments than in NaCl treatments. The germination in PEG treatments was strongly compromised at an osmotic potential of -0.6 MPa, and there was no further seedling growth. In addition, it was observed that shoots were

more adversely affected than roots in salt and drought stresses caused by NaCl and PEG_{6000} treatments. The germination and growth of *L. albus* seedlings were significantly diminished by increasing salt and drought stress, primarily due to drought stress rather than the effects of salt toxicity. Although the NaCl solutions applied in the study had adverse effects on some germination parameters such as mean germination time and germination rate index, these treatments did not have much adverse effects. Therefore, it is recommended that NaCl solutions with lower osmotic potentials be applied for *L. albus* in future studies in order to determine the dose at which germination does not occur.

Ethical Statement

There is no need to obtain permission from the ethics committee for this study**.**

Conflicts of Interest

There is no conflict of interest.

Authorship Contribution Statement

Concept: Beyaz, R., Uslu, V. V.; Design: Beyaz, R.; Data Collection or Processing: Beyaz, R.; Statistical Analyses: Beyaz, R.; Literature Search: Beyaz, R., Uslu, V. V.; Writing, Review and Editing: Beyaz, R., Uslu, V. V.

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