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Effect of NaCl-induced Salt Stress at Germination and Early Seedling Growth Stage in *Lupinus albus* L.

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ABSTRACT

Salinity is a primary abiotic factor affecting agricultural productivity in arid and semiarid environments. The stages that are most vulnerable to salinity are germination and early seedling growth. There are limited reports on the responses of *Lupinus albus* L. to salinity. Therefore, in the study, we aim to test germination and early seedling growth of *L. albus* under different salinity levels. To this end, seeds of *L. albus* were treated with different concentrations (0, 50, 100, 150, and 200 mM) of NaCl under laboratory conditions. A total of 16 parameters, including germination and growth, were examined. The results showed that under 200 mM NaCl, germination percentage (GP) decreased by 13.4% and germination rate index (GRI) decreased by 69.24%, while mean germination time (MGT) increased by 30.02%. In addition, the shoot length (SL), root length (RL), shoot fresh weight (SFW), root fresh weight (RWF), and root dry weight (RDW), root to shoot dry matter (R/S DM), shoot water content (SWC), root water content (RWC), and seedling vigor index (SVI) were reduced respectively by 82.69%, 75.65%, 53.30%, 70%, 66.66%, 70.86%, 23.47%, 0.35% and 82.57% under 200 mM NaCl, compared to the control condition. However, root to shoot ratio (R/S), shoot dry weight (SDW), shoot dry matter (SDM), root dry matter (RDM) were increased by 43.33%, 65.07%, 249.68%, and 3.22% under 200 mM NaCl. Overall, the study results showed that the critical level to mitigate the negative effect of salinity is 150 mM NaCl (-0.6 MPa osmotic potential) for germination and 50 mM NaCl (-0.2 MPa osmotic potential) for growth. Therefore, *L. albus* has a low tolerance to salinity.

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Introduction

Salinity restricts the dispersal of plants throughout their natural habitats and poses a serious agricultural challenge in many parts of the world (Fernandes et al., 2004). An important environmental issue impacts about 20% (954 million hectares) of agricultural land. The Global Map of Salinity-affected Areas The spatial distribution of salt-affected soils indicates that more than 424 million hectares of topsoil and 833 million hectares of subsoil are affected by salinity. Most salt-affected soils are saline, including 85% of topsoils and 62% of subsoils, while sodic soils account for 10% of topsoils and 24% of subsoils. Significantly almost two-thirds of these salt-affected soils are located in arid and semi-arid regions, comprising 37% in deserts and 27% in arid steppe environments (Singh 2021; FAO 2022; Farooq et al. 2024). The principal salt responsible for salt stress is sodium chloride (NaCl). A diverse array of wild plants is adversely affected by sodium ions, while specific species suffer from elevated chloride concentrations. The germination of seeds and the growth of seedlings are the stages most susceptible to salinity. Salt stress induces detrimental physiological and biochemical alterations that impede seed germination. Seed germination and stand establishment can be affected by oxidative stress, osmotic stress, and ion-specific effects. Salinity hinders seed germination by affecting the mobilization of stored reserves, diminishing water availability, and changing protein structural order (Ibrahim, 2016).

In agriculture, the Leguminosae family is the second most significant family behind the Gramineae (Ozaktan et al. 2023). White or field lupin (*Lupinus albus* L.) is a plant of the Leguminosae family that was domesticated as a pulse crop for human consumption and livestock feed before 2000 BC (Ihsan et al. 2024). Over 400 species are recognized within the genus *Lupinus*, of which only four hold agronomic significance: *L. albus* (white lupin), *L. angustifolius* (blue or narrow-leafed lupin), *L. luteus* (yellow lupin), and *L. mutabilis* (pearl or Tarrwi lupin). The initial three species are native to the Mediterranean region, encompassing Türkiye (Al-harbi et al. 2014). *Lupins* are grown on an area of 1 million hectares globally, yielding a total production of 1.385 million metric tons (Ihsan et al. 2024). The elevated protein concentration in their seeds renders them a potential alternative to soybean [*Glycine max* (L.) Merr.] in cattle and poultry feed. White lupin is a versatile, climate-resilient pulse crop with an extraordinarily high protein content, rendering it a viable substitute for soybean in livestock feed. The alkaloid concentration in bitter cultivars varies from 5 to 40 g/kg, whereas low-alkaloid cultivars exhibit levels between 0.08 and 0.12 g/kg. Alkaloid-free cultivars of white lupins exist, and the creation of these mutants has facilitated the use of white lupins as a protein source for animals. White lupins possess moderate to high levels of crude protein (202–424 g/kg), crude fat (60–130 g/kg), and fiber (105–162 g/kg) (David et al. 2024). It can grow very well in marginal areas, but despite all these important features, it is reported that its tolerance to salinity is low (Ihsan et al. 2024). However, there are limited reports (Yu and Rengel, 1999; Fernandes et al., 2004; Slabu et al., 2010; Hussien, 2022) on the effects of salinity on species of *Lupinus* genus, including *L. albus*. Therefore, this study aimed to reduce the knowledge gap on early responses of *L. albus* to salt stress. This study aimed to evaluate the germination and early seedling growth of *L. albus* under different salt stress levels in a laboratory setting.

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.

Methods

Germination tests and morphological observations

Test solutions were formulated using distilled water at concentrations of 0 (control), 50, 100, 150, and 200 mM NaCl (Sigma-Aldrich). Four replicates of 25 seeds were germinated between three rolled filter sheets using 10 mL of the corresponding test solutions. Before planting, seeds were given a fungicide treatment (Thiram 80%), and papers were changed every two days to minimize salt buildup (Beyaz et al., 2011). 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 an incubator in the dark. 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).

Germination percentage (GP) was calculated according to Al-Enezi et al. (2012). (GP) = (Number of germinating seeds/ Total number of seeds) × 100 (Eq.1). The mean time to germination (MGT) was determined by Ellis and Roberts in 1980 MGT=∑Dn/∑D (Eq. 2), where 'n' represents the quantity of germinated seeds on day T, and Σn denotes the cumulative sum of germinated seeds. The speed of germination serves as an index for the germination rate (GRI), determined using the following equation (Maguire 1962): GRI = Σ No of Germinated Seeds/ Σ No of Days (Eq. 3). Seedlings with stunted primary roots and short, thick, spiral-shaped hypocotyls were deemed to have aberrant germination.

Early 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). The Seedling Vigor Index (SVI) was computed using the method of Abdul-Baki and Anderson (1973). (SVI) = (average root length + average hypocotyl length) × germination percentage (GP) (Eq. 4). TheWC = (Fresh weight − Dry weight) / Fresh weight × 100 (Eq. 5) (Zheng et al., 2008); as well as dry matter (DM) = (Dry weight / Fresh weight) × 100 (Eq. 6) (Bres et al., 2022), were calculated using these formulas.

Statistical Data Analysis

The design used was a 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. An analysis of variance (ANOVA) and Duncan's multiple range test procedure were used to investigate potential treatment differences ($P \leq 0.05$). Data expressed as percentages, underwent arcsine transformation of the square root of X prior to statistical analysis (Snedecor and Cochran, 1967). The standard error was computed for each treatment.

Results and Discussion

Effects of Salt Stress on Germination

A crucial step in the establishment of seedlings and subsequent plant health and vigor is seed germination (Cokkızgın, 2012). Rapid seed germination and stand establishment are key factors influencing crop productivity in stressful situations (Ibrahim, 2016; Benlioğlu et al., 2024). One of the most significant variables contributing to delayed seed germination and limiting final germination percentage is salinity (Cokkızgın, 2012). In salty soils, most crops will not germinate. Seed germination is the most crucial stage in the plant life cycle and is sensitive to salinity (Zhang et al. 2024). In this study, the effect of five different levels of NaCl-induced salt stress on the germination parameters of *L. albus* L. seed was investigated under laboratory conditions. The evolution of the early seed germination parameters according to different NaCl treatments is shown in Table 1.

The effect of increasing concentrations of NaCl on germination percentage (GP), mean germination time (MGT), and germination rate index (GRI) of lupin seeds is statistically significant (Table 1). Due to increasing NaCl concentrations, GP and GRI values decreased while MGT values increased. GP values (100%) did not change from 0 to 150 mM, but decreased at 200 mM (86.6%). When the control group and the highest salt stress level (200 mM NaCl) were compared, it was determined that MGT increased by 30.02% and GRI decreased by 69.24%.

Table 1. Impact of different NaCl concentrations on germination

Treatments (mM)	GP	MGT	GRI
	---%---	---day---	---%---
0	100.0±0.0a	4.13±0.13c	6.47±0.75a
50	100.0±0.0a	4.27±0.17c	5.39±1.28a
100	100.0±0.0a	4.43±0.18bc	4.82±0.99b
150	100.0±0.0a	5.05±0.44ab	2.62±1.16b
200	86.6±11.5b	5.37±0.55a	1.99±1.07b
Means	97.32	4.65	4.25
Summary of one-way-ANOVA			
NaCl	*	**	**

*significant at p≤0.05, **significant at p≤0.01. Different letters in the same column signify substantial changes at the 0.05 level. GP: Germination percentage, MGT: mean germination time, GRI: germination rate index

Table 2. Impact of different NaCl concentrations on seedling growth of 21-day-old *L. albus* seedlings.

T	SL	RL	R/S	SFW	RFW	SDW	RDW
	----------cm----------		---%---	--------------------------mg/plant--------------------------			
0	13.35±1.67a	8.05±1.82a	0.60±0.09c	2.42±0.24a	0.30±0.03a	0.189±0.06c	0.030±0.004a
50	7.75±2.41b	4.83±1.25b	0.64±0.21bc	1.56±0.30b	0.22±0.05ab	0.224±0.02bc	0.026±0.008ab
100	5.40±0.80ab	3.93±0.90ab	0.75±0.11abc	1.30±0.08bc	0.15±0.03bc	0.237±0.01b	0.017±0.003bc
150	4.13±1.81bc	3.60±1.05ab	0.91±0.07a	1.14±0.10c	0.12±0.04c	0.257±0.01b	0.013±0.007c
200	2.31±0.38c	1.96±0.37c	0.86±0.28ab	1.13±0.07c	0.09±0.02c	0.312±0.02a	0.010±0.003c
Means	6.59	4.47	0.75	1.51	0.18	0.233	0.019
Summary of one-way-ANOVA							
NaCl	**	**	*	**	**	**	**

T: Treatments (mM); *significant at p≤0.05, **significant at p≤0.01. Different letters in the same column signify substantial changes at the 0.05 level. SL: shoot length, RL: root length, R/S: root to shoot ratio, SFW: shoot fresh weight, RFW: root fresh weight, SDW: shoot dry weight, RDW: root dry weight

Table 3. Impact of different NaCl concentrations on seedling growth of 21-day-old *L. albus* seedlings.

T	SDM	RDM	R/S DM	SWC	RWC	SVI
	---------------------------------------%---------------------------------------					
0	7.87±0.92d	9.92±0.20b	1.27±0.15a	94.70±3.64a	90.07±0.20a	2140±326a
50	14.74±3.44c	11.50±0.84a	0.81±0.17b	85.25±3.44b	88.49±0.27b	1258±300b
100	18.31±0.75bc	11.33±0.24a	0.62±0.03bc	81.68±0.75bc	88.66±0.24b	933±64bc
150	22.77±2.97ab	10.42±0.21b	0.47±0.17cd	77.22±2.9cd	89.57±0.21a	773±271cd
200	27.52±2.39a	10.24±0.59b	0.37±0.00d	72.47±2.39d	89.752±0.43a	373±80d
Means	18.24	10.68	0.71	82.26	89.31	1095
Summary of one-way-ANOVA						
NaCl	**	**	**	**	**	**

T: Treatments (mM); **significant at p≤0.01. Different letters in the same column signify substantial changes at the 0.05 level. SDM: shoot dry matter, RDM: root dry matter, R/S DM: root to shoot dry matter ratio, SWC: shoot water content, RWC: root water content, SVI: seedling vigor index

It was observed that the germination parameters for Lupinus albus L. were sharply affected by 200 mM NaCl. The present findings are consistent with those made by Cokkızgın (2012), who noted that an increase in salt causes a decrease in the proportion of germinating seeds and a delay in the beginning of the germination process in significant legumes like *Phaseolus vulgaris* L.

Reports indicate that elevated salt content diminishes germination percentage and prolongs germination duration (Jamil et al. 2005; Patade et al. 2011; Rouhi et al. 2011; Ansari and Sharif-Zadeh 2012; Beyaz et al. 2018). The reduction in germination rates under elevated salt concentrations may result from impaired water absorption, the toxic impact of certain ions, or the inactivation of essential enzymes required for germination (Aydinşakir et al. 2013). Salt stress impedes seed germination in plants by somehow leading to or enhancing the hydrolysis of urea produced by arginine (Liang and Jiang 2024).

Effects of salt stress on morphological characters

Another stage that is most sensitive to salinity after germination in plants is the early seedling growth stage. The statistical data showed that the effect of increasing concentrations of NaCl on growth parameters such as SL, RL, R/S, SFW, RFW, SDW, RDW, SDM, RDM, R/S DM, SWC, RWC, and SVI is statistically significant (P≤0.01) (Table 2 and Table 3). The SL, RL, SFW, RFW, and RDW were reduced, respectively, by 82.69%, 75.65%, 53.30%, 70%, and 66.66% when treated with 200 mM NaCl, compared to the control. Increasing salt levels had a significant negative effect on early seedling growth of *L. albus* (Figure 1). Similarly, Akladious and Hanafy (2018) reported that 150 and 300 mM NaCl salinity levels caused significant reductions in all growth parameters such as SL, RL, SFW, RFW, SDW, and RDW of *Lupinus termis* L.

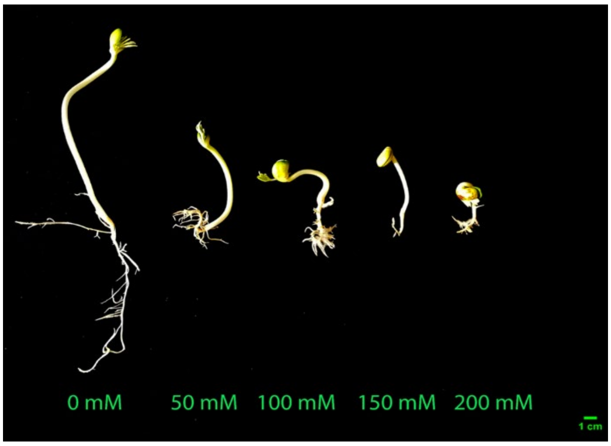

Figure 1. The morphology of 21-day-old seedlings at different NaCl treatments.

Also, Hussien (2022) stated that different growing parameters such as total length of plant, seedling fresh weight, and number of lateral roots were significantly affected by salinity stress (sodium bicarbonate) in *L. albus*. In addition, Slabu et al. (2010) concluded that too much NaCl in the soil affects the vegetative growth of *L. albus* cultivars by decreasing the amount of stored biomass.

Meanwhile, Akladious and Hanafy (2018) stated that the lowest concentration of NaCl (75 mM) caused significant increases in all growth parameters (SL, RL, SFW, RFW, SDW) of *L. termis* as compared with control plants. But the results of the study showed a decrease in these growth parameters (except SDW), beginning with the lowest concentration of NaCl (50 mM) applied to *L. albus*. However, SDW increased with increased salinity levels. Therefore, the results indicated that increased salinity does not always have a negative effect on growth, of different plant organs. Similarly, this was previously approved by Hussien (2022) who concluded that 0.02 M salinity (sodium bicarbonate) increased the fresh weight of 14-day-old *L. albus* seedlings. Also, this agrees with Delgado and Sanchez-Raya (2007), who proved that increasing the salt content does not necessarily harm certain plant organs in sunflower. In addition, according to Yu and Rengel (1999), NaCl concentrations (12.5, 25, 50, and 100 mM) had no effect on SFW and RWF of *L. angustifolius* seedlings; however, increasing NaCl concentrations had an effect on RWF and RDW, causing a decrease in their values. Considering the above parameters, overall shoot growth appears to be more affected by salinity than root growth. However, contrary to these results, Yu and Rengel (1999) in *Lupinus angustifolius* L., Van Steveninck et al. (1982) in *Lupinus luteus* and *Lupinus angustifolius*, and Jeschke et al. (1986) in Lupinus albus L. cv 'Ultra' reported that salinity has a more negative effect on root growth, than in many other plants. Responses to salt stress can vary significantly among plants, within the same species, and even among varieties/cultivars and genotypes of depending on their genetic structure or habitats.

In the current study, the results show that salinity caused an increase in SDM and RDM values, and a decrease in SWC and RWC values of *L. albus* seedlings (Table 2). Similar to these data, Beyaz et al. (2011) reported that salinity (NaCl) caused an increase in dry matter in sainfoin (Onobrychis sativa) seedlings. In addition, Bres et al. (2022) stated that due to salinity, the water content in the *Lactuca sativa* L seedlings was decreasing gradually, whereas the dry matter content was increasing. However, when the DM ratios under salt stress were examined, it was observed that the increase in the shoot was higher than in the root. On the other hand, with respect to the WC, it was determined that the decrease in values was more in shoots (Table 2). However, the R/S DM was significantly (P≤0.01) reduced with increasing salt levels. According to Beyaz et al. (2016), the decrease in plant tissue water content increases the dry matter ratio in the tissues. Therefore, more dry matter accumulation in the shoots of seedlings may be explained in this way. Salinity typically diminishes the growth of crop shoots more significantly than root growth, as assessed by dry weight rather than length measurements (Shalhevet et al. 1995). These study data showed that salinity affected roots more adversely than shoots in terms of dry weight in seedlings. It has also been reported in previous studies, that salinity affects the roots more negatively than shoots in plants (Kaya et al. 2003; Bandeoğlu et al. 2004).

Salinity significantly (P≤0.01) decreased SVI of L. albus, which measures the health of seedlings. This decrease in SVI ranged from 2140 to 373 when compared to control and 200 mM (Table 2). The fact that the seedling vigor index decreased as salinity increased suggests that seeds are negatively impacted by salt content (Khan et al., 2022). In addition, the absorption of essential nutrients like phosphorus (P) and potassium (K) is inhibited by salt, which has been observed to have an adverse effect on seedling growth and vigor index (Nasim et al., 2008). Because the seedling vigor index can be determined from root and shoot length, it is the most accurate predictor of a plant's resistance to salt. Consequently, the seedling vigor index can be used to gauge how well plants tolerate salt (Zhang et al. 2021). It has also been reported in previous studies that salinity affects and reduces SVI in other plants as well (Chakraborty et al. 2019; Rajabi Dehnavi et al. 2020).

Conclusion

In conclusion, this study demonstrated that the early seedling growth stage of *L. albus* is more sensitive to salt stress than the germination stage. This sensitivity started at 50 mM NaCl (-0.2 MPa) for the early seedling growth stage, and 150 mM NaCl (-0.6 MPa osmotic potential) for germination. Therefore, in the light of these data, it is concluded that growing *L. albus* in areas where it would be exposed to 50 mM and above salt (NaCl) stress would result in significant yield losses.

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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