Journal of Tekirdag Agricultural Faculty
Tekirdağ Ziraat Fakültesi Dergisi

Ocak/January 2024, 21(1)
Başvuru/Received: 21/12/22
Kabul/Accepted: 10/07/23
DOI: 10.33462/jotaf.1222317

http://dergipark.gov.tr/jotaf
http://jotaf.nku.edu.tr/

ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Effect of NaCl-induced Salt Stress on Germination and Initial Seedling Growth of *Lotus corniculatus* L. cv. 'Leo'

NaCl Kaynaklı Tuz Stresinin *Lotus corniculatus* L. cv. 'Leo'nun Çimlenmesi ve İlk Fide Büyümesi Üzerindeki Etkisi

Ramazan BEYAZ[1*], Ahmet KAZANKAYA[2]

Abstract

Lotus corniculatus L. is one of the agronomically and economically important perennial legume forage species with moderately salt-tolerant. It is well known that even the cultivars of the same species in plants have different responses in salinity. However, studies on the salt response of *L. corniculatus* and its cultivars, which are more advantageous than other forage crops such as white clover (*Trifolium repens*) and alfalfa (*Medicago sativa* L.), in the use of marginal agricultural lands affected by abiotic stress factors such as salinity, are limited. Under salt stress, the most crucial phases of the plant life cycle that are directly related to the survival of the plant are seed germination, growth, and vigour. Therefore, this study was carried out to determine the germination and growth responses of *L. corniculatus* cultivar 'Leo', which is known to have higher tannin content than other cultivars, under NaCl-derived salt stress in vitro. For this purpose, *L. corniculatus* seeds were cultured in MS (Murashige and Skoog/Gamborg) medium containing 0, 40, and 80 mM NaCl for 14 days. Seed germination percentage, mean germination time, germination rate index, shoot-root length, root to shoot length ratio, shoot-root fresh dry weight, shoot-root dry matter, the ratio of root to shoot dry matter, shoot-root water content and seedling vigour index parameters were measured. According to the results of the research, the germination percentage did not change in the applied NaCl treatments, but the germination rate decreased. However, shoot length decreased and root length increased. Although there was no statistically significant change in shoot and root fresh-dry weight, both decreased in 80 mM NaCl treatment. The shoot and root dry matter increased and the water content decreased. Also, the seedling viability index decreased. In 40 mM NaCl treatment, on the other hand, there was an increase in shoot fresh-dry weight, dry matter ratio and seedling viability index with the positive effect of low dose. Within the scope of this study, comprehensive information was presented for *L. corniculatus* (cultivar 'Leo'), an important forage plant, in terms of germination and seedling growth under salt stress.

Keywords: *Lotus corniculatus* L., Salinity, Germination, Initial seedling growth stage, *In vitro*

[1*] **Sorumlu Yazar/Corresponding Author:** Ramazan Beyaz, Kırşehir Ahi Evran University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, Kırşehir, Türkiye. E-mail: ramazanbeyaz@gmail.com OrcID: 0000-0003-4588-579X

[2] Ahmet Kazankaya, Kırşehir Ahi Evran University, Faculty of Agriculture, Department of Horticulture, Kırşehir, Türkiye. E-mail: ahmeta.kazankaya@ahievran.edu.tr OrcID: 0000-0002-1081-4281

Atıf: Beyaz, R., Kazankaya, A. (2024). NaCl kaynaklı tuz stresinin Lotus corniculatus L. cv. 'Leo'nun çimlenmesi ve ilk fide büyümesi üzerindeki etkisi. Tekirdağ Ziraat Fakültesi Dergisi, 21(1): 24-34.

Citation: Beyaz, R., Kazankaya, A. (2024). Effect of NaCl-induced salt stress on germination and initial seedling growth of Lotus corniculatus L. cv. 'Leo'. *Journal of Tekirdağ Agricultural Faculty*, 21(1): 24-34.

Öz

Lotus corniculatus L. orta derecede tuza toleranslı, tarımsal ve ekonomik açıdan önemli çok yıllık baklagil yem türlerinden biridir. Bitkilerde aynı türün çeşitlerinin bile tuzluluğa farklı tepkiler verdiği iyi bilinmektedir. Ancak tuzluluk gibi abiyotik stres faktörlerinden etkilenen marjinal tarım arazilerinin kullanımında ak üçgül (*Trifolium repens*) ve yonca (*Medicago sativa* L.) gibi diğer yem bitkilerine göre daha avantajlı olan *L. corniculatus* ve çeşitlerinin tuz tepkisi üzerine yapılan çalışmalar sınırlıdır. Tuz stresi altında, bitkinin hayatta kalmasıyla doğrudan ilgili olan bitki yaşam döngüsünün en önemli aşamaları tohumların çimlenmesi, büyümesi ve canlılığıdır. Bu nedenle bu çalışma, diğer çeşitlere göre daha yüksek tanen içeriğine sahip olduğu bilinen *L. corniculatus* çeşidi 'Leo'nun NaCl türevli tuz stresi altındaki in vitro çimlenme ve büyüme tepkilerini belirlemek amacıyla yapılmıştır. Bu amaçla *L. corniculatus* tohumları 0, 40 ve 80 mM NaCl içeren MS (Murashige ve Skoog/Gamborg) besiyerinde 14 gün kültüre edilmiştir. Tohum çimlenme yüzdesi, ortalama çimlenme süresi, çimlenme oranı indeksi, sürgün-kök uzunluğu, kök-sürgün uzunluğu oranı, sürgün-kök yaş-kuru ağırlığı, sürgün-kök kuru maddesi, kök-sürgün kuru madde oranı, sürgün- kök su içeriği ve fide canlılık indeksi parametreleri ölçülmüştür. Araştırma sonuçlarına göre, uygulanan NaCl uygulamalarında çimlenme yüzdesi değişmemiş ancak çimlenme hızı azalmıştır. Bununla birlikte sürgün uzunluğu azalmış, kök uzunluğu artmıştır. İstatisitiki açıdan sürgün ve kök yaş-kuru ağırlığında önemli bir değişme olmamasına rağmen, 80 mM NaCl uygulamasında her ikisinde de azalış olmuştur. Sürgün ve kök kuru madde oranı artmış su içeriği azalmıştır. Ayrıca, fide canlılık indeksi de azalmıştır. 40 mM NaCl uygulamasında ise düşük doz olumlu etkisi ile sürgün yaş-kuru ağırlık, kuru madde oranı ve fide canlılık indeksinde artış olmuştur. Bu çalışma kapsamında önemli bir yem bitkisi olan *L. corniculatus* (kültivar 'Leo') için tuz stresi altında çimlenme ve fide gelişimi bakımından kapsamlı bilgi sunulmuştur.

Anahtar Kelimeler: *Lotus corniculatus* L., Tuzluluk, Çimlenme, İlk fide büyüme aşaması, In vitro

1. Introduction

Worldwide, millions of hectares of land are too saline to support commercial crop yields, and more land is rendered unproductive every year due to salt buildup. Problems with salinity in agriculture are often limited to dry and semiarid areas when rainfall is insufficient to move salts out of the root zone of the plant (Carter, 1975). When there is an excess of sodium chloride, sodium carbonate, sodium sulfate, or salts of magnesium, it is considered to be a salinity problem since the effect gets more pronounced the more excess there is (Chapman, 1975). Salts like chloride and sulphate make up the majority of soils and groundwater in nature (Tarchoune et al., 2010). The primary salt that causes salt stress is typically NaCl. A wide variety of wild plants are poisoned by sodium ions, whereas certain plants are harmed by high chloride concentrations. Because the low potassium level induced by absorbing more sodium hinders plant growth, plants engage their high affinity system for uptaking potassium when sodium enters the cytoplasm in order to absorb enough of this ion (Azarafshan and Abbaspour, 2014). Saline soil is any soil with water-soluble salt concentrations more than 4 dS m^{-1} (Shokat and Großkinsky, 2019). However, the saline area is divided into four main types based on its salinity, namely very severely salinized (ECe> 16 dS m^{-1}), severely salinized (ECe 8-16 dS m^{-1}), moderately salinized (ECe 4-8 dS m^{-1}), and mildly salinized (ECe 2-4 dS m^{-1}) (Dornburg et al., 2011). Salt harms plants by creating osmotic stress, ion imbalance, and oxidative damage. Plants have developed highly complex processes, including osmotic stress resistance, ion exclusion, and tissue tolerance, in order to survive salt stress (Wang et al., 2021). However, salt stress has an impact on plants by limiting their ability to absorb water, rupturing their biological membranes, causing ionic imbalance, oxidative damage, and nutritional imbalance, slowing down cell division and growth, reducing the rate of photosynthesis, altering their lipid metabolism, and affecting their yield characteristics. Salinity has a significant impact on seed germination, one of the most important stages of plant development (Jaleel et al., 2007). However, the most delicate and crucial stages of most plants' establishment in saline settings are during their seedling establishment (Çakmakçı and Dallar, 2019; Wang et al., 2019; Altuner et al., 2022).

Like many other cultivated plants and/or crops, legume family members, including *Lotus corniculatus* L. (also known Bird's-foot trefoil, BFT), are adversely affected by salinity. *L. corniculatus* a perennial legume forage is regarded as one of the most important forage plants in terms of agriculture due to its many benefits, including its ability to grow in low fertile, acidic, and its anti-bloating properties due to its tannin content, and it is also frequently used to stop roadside erosion (Wang et al., 2021). In temperate places of the world, *L. corniculatus* may eventually take the place of white clover and alfalfa (Savić et al., 2019). Despite all these important features, most *L. corniculatus* are sensitive to salinity (Bao et al., 2014). Under conditions of salt stress, seed germination behavior varies from plant species to species and also significantly from cultivar to cultivar (Munns and Tester, 2008). 'Leo' has a very good seedling vigour and high yielding cultivar (Undersander et al., 1993), but the comprehensive responses to salt stress are not reported yet about this cultivar. There is also a gap in our knowledge in germination and early seedling growth. Therefore, the primary goal of this study was to investigate the effects of salt stress on seed germination and early seedling growth in *L. corniculatus* cultivar 'Leo' under in vitro conditions.

2. Materials and Methods

2.1. Plant Material

In this study, the seeds of the *Lotus corniculatus* L. cv. 'Leo', harvested in 2022 and supplied by Utah State University, Plants, Soils and Climate Department, were used as plant material.

2.2. Plant Tissue Culture and SaltTreatments

The *L. corniculatus* seeds were surface sterilized in 50% commercial bleach (Clorox-USA, containing 8.25% sodium hypochlorite) in which 1 drop of Tween-20 (Acros Organics) was added for 25 minutes and then rinsed 3 times with distilled water. Sterilized seeds were sown on standard MS (Murashige and Skoog/Gamborg) (Gamborg et al., 1968), Plant Media, USA) medium containing 3% sucrose (Research Product International RPI, USA) and 7% agar (Plant Media, USA). For salt stress, seeds were sown in standard MS/Gamborg medium with 40 and 80 mM NaCl. Before autoclaving at 121°C, 7.25 psia for 20 minutes, the pH of the medium was adjusted to 5.7 with 1 M NaOH or HCl. Germination of seeds and subsequent seedling development were carried out at 25±1°C under white fluorescent lamps at an intensity of 30 µmol m^{-2} s^{-1} (PAR) in a photoperiod of 16h light and 8h dark.

2.2. Germination and Growth

When the growing radicle elongated to 0.2 cm, the seed was considered germinated. For 14 days, the proportion of seeds that germinated was recorded every 24 hours (ISTA, 2003). Mean germination time (MGT) was calculated according to Ellis and Roberts (1980). $\mathrm{MGT} = \sum \mathrm{Dn}/\sum \mathrm{D}$, 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 percentage of seeds that germinated after being exposed to salt stress were estimated using the equation:

$$\text{Germination percentage (GP)} = (\text{Number of germinating seeds}/\ \text{Total number of seeds}) \times 100$$

(Al-Enezi et al., 2012) (Eq.1).

Growth parameters (length (cm) of shoot and root; fresh and dry weight (mg) of shoot and root) were measured in 14-day-old seedlings (*Figure 1*.). Dry weights were calculated after samples were dried in an oven (VWR Scientific Inc., USA) at 70°C for 48 hours (Beyaz et al., 2011). Water content (WC), dry matter (%) (DM) and vigor index (VI) were calculated according to the following formulas, respectively:

$\text{Water content (WC)} = (\text{fresh weight} - \text{dry weight})/\text{fresh weight} \times 100$ (Zheng et al., 2008) (Eq.2).

$\text{Dry matter (DM)} = (\text{dry weight}/\text{fresh weight}) \times 100$ (Bres et al., 2022) (Eq.3).

$$\text{Vigor index (VI)} = (\text{average root length} + \text{average hypocotyl length}) \text{ x germination percentage (GP)}$$

(Abdul-Baki and Anderson, 1973) (Eq.4).

Germination rate was expressed as the germination rate index (GRI) according to Maguire (1962):

$\mathrm{GRI} = \Sigma \text{ No of Germinated Seeds}/\Sigma \text{ No of Days}$ (Eq.5).

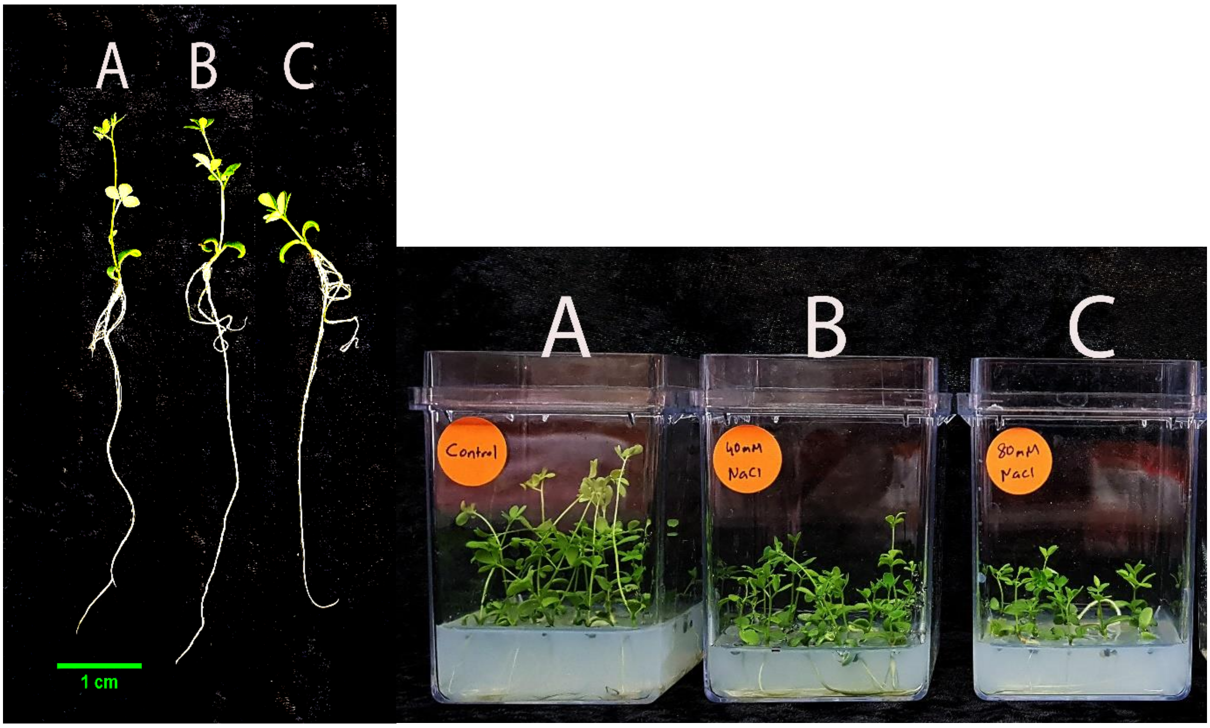


Figure 1. 14-days-old seedlings of Lotus corniculatus L. cultivar 'Leo' at different NaCl concentrations under in vitro conditions. A)0 mM NaCl-control- B) 40 mM NaCl C) 80 mM NaCl.

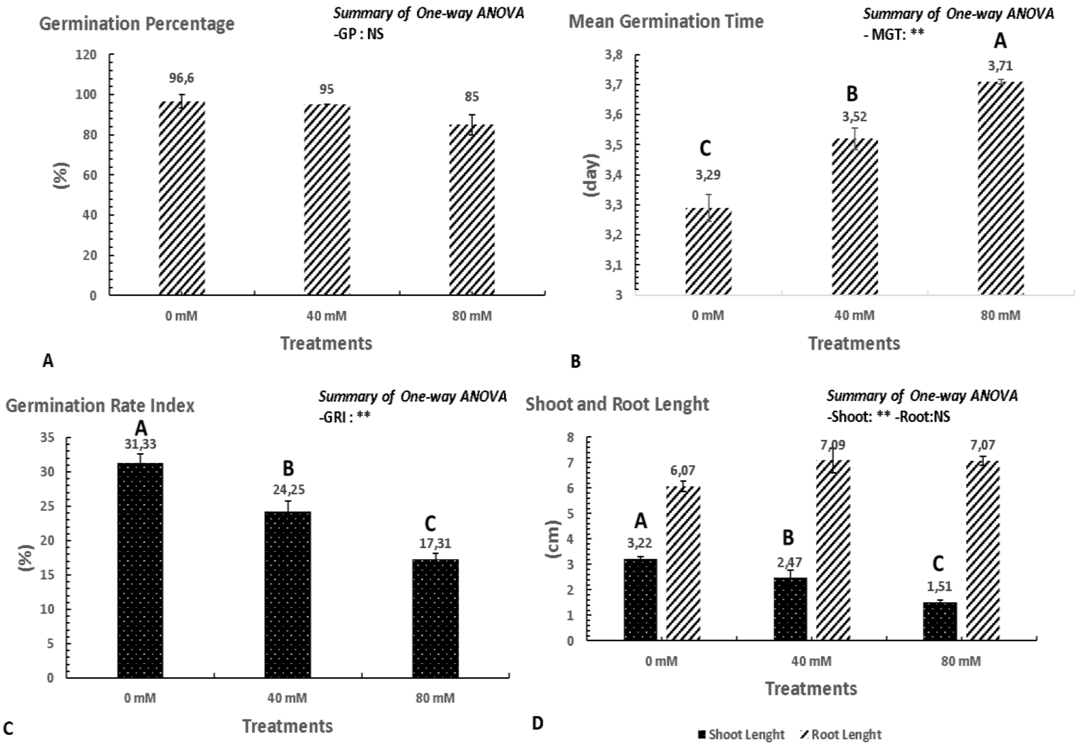
2.2. Statistical Analysis

The study was carried out as a completely randomized design with 3 replications. For each treatment, a one-way ANOVA was done using the SPSS statistical program (Version 22). The means were compared using Duncan multiple range test at $p<0.05$. Before statistical analysis, the data in percentages were transformed using Arcsine transformation (Snedecor and Cochran, 1967).

3. Results

NaCl-induced salt stress had a significant ($p<0.01$) impact on mean germination time and germination rate index (speed of seed germination) but no noticeable effect on germination percentage (*Figure 2A-2B-2C*). The control had the highest percentage of seeds that germinated (96.6%), whereas the 80 mM NaCl treatment had the lowest percentage of seeds that germinated (85%). Under control, 40 mM NaCl, and 80 mM NaCl treatments, the mean germination time was measured at 3.29 days, 3.52 days, and 3.71 days, respectively. Germination rate index was recorded at 31.33%, 24.25% and 17.31% under control, 40 mM NaCl and 80 mM NaCl treatments, respectively. Overall, the germination percentage and germination rate index were reduced by 1.65% and 12.00%, 40% and 44.74%, under 40 mM NaCl and 80 mM NaCl treatments, respectively. On the orher hand, mean germination time was incresed by 6.99% and 12.76% under 40 mM NaCl and 80 mM NaCl treatments, respectively.

The shoot length was statistically significantly ($p<0.01$) affected by the NaCl induced salt stress, while the root length was not affected (*Figure 2D*). Maximum shoot lenght (3.22cm) was recorded in the control, while the lowest shoot lenght (1.51cm) was recorded for 80 mM NaCl treatment. However, root to shoot ratio was significantly ($p<0.01$) affected by NaCl treatments (*Figure 2E*). Maximum root to shoot ratio (1.88%) was recorded in the control, while the lowest shoot lenght (4.68%) was recorded for 80 mM NaCl treatment. Overall, as compared to control, shoot lenght was reduced by 23.29% under 40 mM NaCl, and 53.10% under 80 mM NaCl treatments. However, root to shoot ratio was increased by 52.65% under 40 mM NaCl treatment, and 148.93% under 80 mM NaCl treatment, in comparison with control. Nonsignificant differences were observed among NaCl treatments with respect to shoot and root fresh weight and shoot and root dry weight (*Figure 2F-2G*). However, a significant decrease was observed in total shoot and root fresh weights, depending on the levels of NaCl treatments. Total shoot and root wet weight were recorded as 0.130 mg per plant in the control group, 0.128 mg per plant in the 40 mM NaCl treatment, and 0.106 mg per plant in the 80 mM NaCl treatment. Likewise, a significant decrease was noted in total shoot and root dry weights (excluding 40 mM NaCl treatment) due to the levels of NaCl treatments.



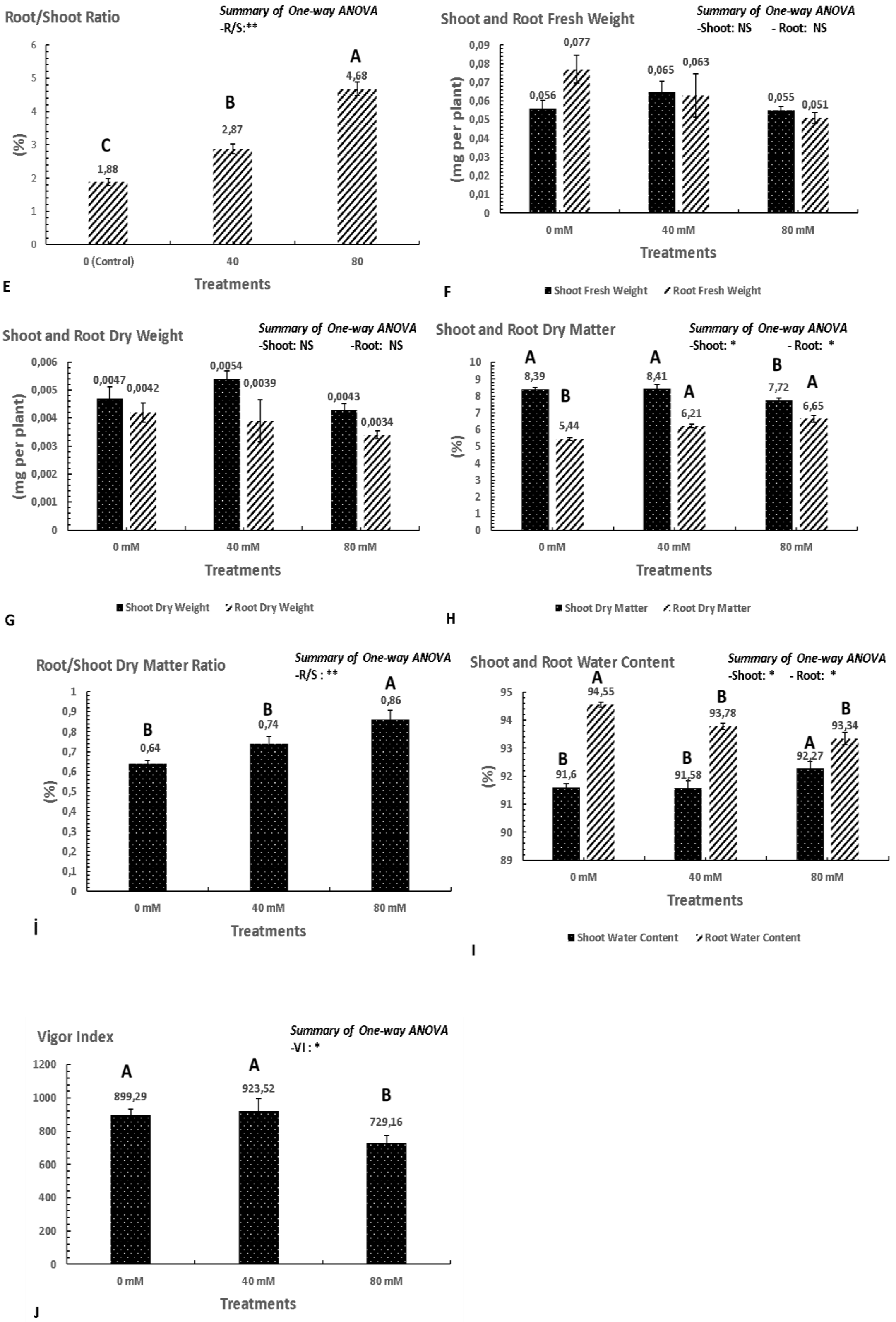


Figure 2. The effect of different levels (0, 40, and 80 mM NaCl) of the NaCl-induced salt stress on germination percentage, mean germination time, germination rate index, shoot and root length, root/shoot ratio, shoot and root fresh weight, shoot and root dry matter, the ratio of root to shoot dry matter, shoot and root water content, and vigor index of 14-days-old BFT seedling. **: p<0.01; *: p<0.05; NS: non-significant.

Total shoot and root wet weight were recorded as 0.0089 mg per plant in the control group, 0.0093 mg per plant in the 40 mM NaCl treatment, and 0.0077 mg per plant in the 80 mM NaCl treatment. Significant differences were found among NaCl treatments in terms of shoot and root dry matter, and also the ratio of root to shoot dry matter (*Figure 2H-2İ*). 8.39%, 8.41% and 7.72% values for shoot dry matter were recorded in control, 40 mM NaCl treatment and 80 mM NaCl treatment, respectively. For root dry matter, the values of 5.44%, 6.21% and 6.65% were recorded in control, 40 mM NaCl treatment and 80 mM NaCl treatment, respectively. The values of 13.83%, 14.62% and 14.37% were recorded in control, 40 mM NaCl treatment and 80 mM NaCl treatment, respectively, when shoot and root dry matter were collected together. Shoot to root dry matter ratio and NaCl treatments showed significant (p<0.01) variations (*Figure 2İ*). Values of 0.64%, 0.74% and 0.86% were recorded in control, 40 mM NaCl treatment and 80 mM NaCl treatment, respectively. In comparison to the control, the ratio of root to shoot dry matter increased by 15.62% under 40 mM NaCl treatment and by 34.37% under 80 mM NaCl treatment. In terms of shoot and root water content, the NaCl treatments showed significant (p<0.05) variations (*Figure 2I*). The highest water content of shoot (92.27%) was obtained in 80 mM NaCl treatment while the lowest water content of shoot (91.60%) was measured under control. However, the highest water content of root (94.55%) was obtained in control while the lowest water content of shoot (93.34%) was measured under 80 mM NaCl treatment. Under 80 mM NaCl treatment, the shoot water content rose by 0.73% in comparison to control. The root water content decreased by 1.17% in the 80 mM NaCl treatment compared to the control.

4. Discussion

Due to the increase in salt of agricultural soils, forage legumes experience yield losses and low nutritional status (Savić et al., 2019). Although salt stress impacts a plant's growth at every stage, most plant species are known to be particularly vulnerable at the seed germination and seedling growth stages (Bybordi, 2010). The study's findings showed that the impacts of salinity levels were significant for mean germination time and germination rate index (germination speed) but not for germination percentage (*Figure 2A-2B-2C*). Specifically, 80 mM NaCl treatment prolonged the mean germination time by 12.7%, and slowed the germination speed by 44.74%. Ion imbalance, osmotic regulatory issues, and finally a decrease in seed water uptake are all caused by high salinity levels (Moss and Hoffman, 1977). Lotus corniculatus is also considered as a germplasm source for salt-tolerant plants (Ünlüsoy et al., 2023). However, the results of this study showed that seeds of *L. corniculatus* cultivar 'Leo' were significantly affected in terms of germination parameter (excluding germination percentage) performance starting from 80 mM NaCl treatment. It is crucial to test a plant's salt tolerance early on since seeds with a faster rate of germination in salty environments may be expected to establish themselves more quickly, producing larger yields (Petrovıć et al., 2016). Therefore, although there is no study on yield and yield components in this study, it can be interpreted that the negative effects of the germination parameters of the 'Leo' cultivar in 80 mM NaC treatment may also affect its yield. It is known that NaCl treatments between 100 mM (~10 dS/m) and 150 mM (~15 dS/m) of legume forage crops, including *Lotus corniculatus*, have negative effects on germination and seedling growth (Beyaz et al. 2011; Azarafshan and Abbaspour, 2014; Beyaz et al., 2018). However, it is well known that even cultivars within the same species can respond differently to many abiotic stress factors such as salinity due to their genotypic structure. Therefore, testing other cultivars of *Lotus corniculatus* for germination under salinity stress will contribute positively to the emergence of an average salt tolerance level. However, the results of this study confirmed the reports of Cokkızgın (2012), Topçu-Demiroğlu and Özkan (2016) and Bhattarai et al. (2020), that germination parameters such as germination percentage, mean germination time and germination rate index (speed of germination) reduced with the increasing salt stress levels in other legumes. According to Jaleel et al. (2007), at lower salt levels, germination was postponed, while at higher salinity regimes, it was suppressed. Therefore, the NaCl treatments applied in this study can be expressed as the low salinity level for the germination of *L. corniculatus* cultivar 'Leo'.

Salt stress negatively affects the growth parameters of plants depending on the applied level and time. In this study, the applied NaCl treatments had significant an impact on growth metrics such as shoot lenght, root to shoot ratio, shoot and root dry matter, the ratio of root to shoot dry matter, shoot and root water content and seedling vigour index (*Figure 2D-2E-2İ-2I-2H-2J*). On the other hand, applied NaCl treatments for parameters fresh and dry weights of shoots and roots had an effect, but this was not statistically significant (*Figure 2G-2F*). A significant decrease (55.10% at 80 mM NaCl) was observed in shoots and very little increase in roots, depending on the increased salt level. However, when looking at root to shoot ratio, there was a significant increase (148.93% ar 80 mM NaCl). The most common morphological sign of saline damage to a plant is slowed growth as a result of cell elongation inhibition (Nieman, 1965). However, under the salinity conditions, as the root to shoot ratio rises, the need to give components to the shoot decreases, which may boost the root's capacity to do so and provide an adaptation benefit (Cheeseman, 1988; Bernstein, 2013). Salt stress may have reduced shoot growth by impairing the efficiency of photosynthetic product transport and assimilation (Jaleel et al., 2007). According to Bernstein (2013), the reduced ability of the shoot to deliver nutrients to the root and growing tissues is one potential drawback of such a transformation, which is expected to have an impact on plant growth and survival, especially under long-term salinization. Therefore, the decrease in shoot length, increase in root length and root shoot ratio indicates that *L. corniculatus* tries to increase its adaptability at the salinity levels applied in the study. In supporting of our observation, Antonelli et al. (2021) reported that the root to shoot ratio of different *L. corniculatus* cultivars increased depending on the increasing salt level at greenhouse conditions. Similarly, Bandeoğlu et al. (2004) reported that salt stress caused a significant decrease in shoot length of lentil seedlings. Fresh and dry weight of shoot and root are important indicators of salinity tolerance of forage plants. NaCl caused an increase in shoot fresh and dry weight with low concentration and a decrease with the highest concentration (*Figure 2F-2G*). On the other hand, root fresh and dry weight decreased depending on the increasing salt level (*Figure 2F-2G*). It is seen that the root fresh and dry weight decreased more than the shoot. From the results of the research, it is understood that the 'Leo' cultivar shows low tolerance in terms of these parameters, even EC 4-8 dS m^{-1}, which are considered moderate salinity levels and applied. Similar to this research findings, Beyaz et al. (2011) reported that fresh-dry weight decreased due to increasing salinity in sainfoin, another important forage crops, in their study *in vitro* study. Uchiya et al. (2016) stated that root dry weight of *L. corniculatus* seedling decrease depend on increased salinity levels (from 0 to 150 mM). Moreover, Bao et al. (2014) noted that depend on increased salt stress (from 0 to 200 mM) shoot dry weight of *L. corniculatus* seedling decreased. The accumulation of dry matter determines the crop yield. Under stress conditions, changes may occur in the distribution of photosynthesis products in plant organs (such as leaf, stem and root) in order to adapt to the stress factor (Jiang et al., 2018). The accumulated shoot and root dry mass of the crops can also be used to study how different stress treatments affect their growth. The results of this study showed that with increasing salinity, the dry matter ratio in the shoot decreased ($p<0.05$), whereas the dry matter ratio in the root increased ($p<0.05$) (Figure 2H). However, the ratio of root to shoot dry matter increased with increasing salt levels (*Figure 2İ*). The increase in the dry matter ratio in the root under salt stress may be explained by the change in the allocation of biomass to increase the root resistance in stress tolerance. Similarly, Antonelli et al. (2021) reported that both of shoot and root dry matter of *L. corniculatus* cultivar San Gabriel seedlings decrease under salt stress (150 mM NaCl), but this decreasing is more in shoots. Beyaz et al. (2011) stated that dry matter of sainfoin seedlings increase depend on increasing salt stress (from 0 to 30 dS m^{-1}). Teakle et al. (2006) noted that shoots and roots dry matter of *L. corniculatus* cultivar San Gabriel decrase under salt stress (400 mM NaCl). The main effects of stress are a decrease in water content and damage to cellular membranes, which help the body deal with abiotic stress such as salt and drought (Zhou et al., 2012). The results of this study showed that the water content of the shoot increased, while the water content of the root decreased due to increasing salt levels (*Figure 2I*). The root-water-uptake of plants is constrained by salinity stress, which also affects plant growth (Wang et al., 2012). However, it was observed that the decrease in water content in the root was not very severe at the applied salt levels (40 and 80 mM). As one of the reasons, it can be speculated that the applied salt levels do not cause a severe negative effect on the root in terms of water uptake. Seedling vigor index is an important criterion to understand plant’s response to salt stress. In this study, seedling vigour index increased at 40 mM NaCl and decreased at 80 mM NaCl (*Figure 2J*). Generally speaking, we may assume that when plants are exposed to low salt concentrations, the elongation of the stem may cause osmotic adjustment activity, which may enhance growth (Abdul-Qados, 2011). The seedling vigour index was calculated on the basis of seed germination and root and stem (shoot) growth. When shoot and root length are collected together, it is seen that there is an increase at 40 mM compared to the control group (*Figure 2D*). Therefore, it can be said that this increase in 40 mM NaCl is due to the low dose positive effect. In general, it has been reported in the literature (Khajeh-Hosseini et al., 2003; Cokkızgın, 2012; Dehnavi et al., 2020) that the seedling vigor index decreased in other crops due to increased salinity levels, similar to the results of this study.

5. Conclusions

In conclusion, this study demonstrated that applied NaCl treatments did not have a significant effect on the germination percentage of *L. corniculatus* cultivar 'Leo', however, highest NaCl treatment decreased the speed of germination (44.74%). However, 80 mM NaCl treatment resulted in a 53.10% decrease in shoot length and 16.47% increase in a seedling root length. In addition, 80 mM NaCl treatment decreased the total (shoot + root) fresh weight (20.30%), total dry weight (13.48%) and total water content (0.29%) and increased the total dry matter ratio (3.90%) in a seedling. However, it caused a decrease in the seedling survival index (17.15 %). Therefore, when the germination and growth parameters are evaluated cumulatively, it can be said that the cultivar 'Leo', which is a forage plant, suffered a significant loss of yield at the salinity level of 80 mM NaCl (~ 8 dS/m). However, it should also be tested at higher levels. In addition, it is suggested that in future studies, salt stress in nature should be tested for the drought cultivar 'Leo', which is known as the sibling stress factor.

Ethical Statement

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

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

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

References

Abdul-Baki, A. A. and Anderson, J. D. (1973). Vigor determination in soybean seed by multiple criteria. *Crop Science,* 13: 630-633.

Abdul-Qados, A. M. S. (2011). Effect of salt stress on plant growth and metabolism of bean plant *Vicia faba* (L.). *Journal of the Saudi Society of Agricultural Sciences,* 10: 7-15.

Al-Enezi, N. A., Al-Bahrany, A. M. and Al-Khayri, J. M. (2012). Effect of X-irradiation on date palm seed germination and seedling growth. *Emirates Journal of Food and Agriculture,* 24(5): 415-424.

Altuner, F., Oral, E. and Baran, İ. (2022). Determination of the effects of salt (NaCl) stress on germination in some barley (*Hordeum vulgare* L.) varieties. *Journal of Tekirdag Agricultural Faculty,* 19(1): 39-50.

Antonelli, C. J., Calzadilla, P. I., Campestre, M. P., Escaray, F. J. and Ruiz, O. A. (2021). Contrasting response of two *Lotus corniculatus* L. accessions to combined waterlogging–saline stress. *Plant Biology,* 23: 363-374.

Azarafshan, M. and Abbaspour, N. (2014). Growth and physiological parameters under salinity stress in *Lotus corniculatus*. *Iranian Journal of Plant Physiology,* 4(2): 991-997.

Bandeoğlu, E., Eyidoğan, F., Yücel, M. and Öktem, H. A. (2004). Antioxidant responses of shoots and roots of lentil to NaCl-salinity stress. *Plant Growth Regulation,* 42: 69-77.

Bao, A. K., Wang, Y. W., Xi, J. J., Liu, C., Zhang, J. L. and Wang, S. M. (2014). Co-expression of xerophyte *Zygophyllum xanthoxylum* ZxNHX and ZxVP1-1 enhances salt and drought tolerance in transgenic *Lotus corniculatus* by increasing cations accumulation. *Functional Plant Biology,* 41: 203-214.

Bernstein, N. (2013). Effects of Salinity on Root Growth. In: Plant Roots: The Hidden Half, 4th edition, (A. Eshel, T. Beeckman, eds.). CRC. 848 Pages. Boca Raton: CRC Press.

Beyaz, B., Kaya, G., Cocu, S. and Sancak, C. (2011). Response of seeds and pollen of *Onobrychis viciifolia* and *Onobrychis oxyodonta* var. armena to NaCl stress. *Scientia Agricola,* 68(4): 477-481.

Beyaz, R., Yildiz, M. and Sancak, R. (2018). Morphological and biochemical responses of sainfoin (*Onobrychis viciifolia* Scop.) ecotypes to salinity. *Legume Research,* (41): 253-258.

Bhattarai, S., Biswas, D., Fu, Y. B. and Biligetu, B. (2020). Morphological, physiological, and genetic responses to salt stress in alfalfa. *Agronomy,* 10(577): 1-15.

Bres, W., Kleiber, T., Markiewicz, B., Mieloszyk, E. and Mieloch, M. (2022). The effect of NaCl stress on the response of lettuce (*Lactuca sativa* L.). *Agronomy,* (12) 244: 1-14.

Bybordi, A. (2010). The influence of salt stress on seed germination, growth and yield of canola cultivars. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca,* 38(1): 128-133.

Carter, D. L. (1975). Problems of Salinity in Agriculture. Plants in Saline Environments Edited by A Poljakoff-Mayber and J. Gale. Springer-Verlag Berlin Heidelberg Newyork.

Chapman, V. J. (1975). The Salinity Problem in General, Its Importance, and Distribution with Special Reference to Natural Halophytes. Plants in Saline Environments Edited by A Poljakoff-Mayber and J. Gale. Springer-Verlag Berlin Heidelberg Newyork.

Cheeseman, J. M. (1988). Mechanisms of salinity tolerance in plants. *Plant Physiology,* 87: 547-550.

Cokkızgın, A. (2012). Salinity stress in common bean (*Phaseolus vulgaris* L.) seed germination. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca,* 40(1): 177-182.

Çakmakçı, S. and Dallar, A. (2019). Effects of Different Temperatures and Salt Concentrations on the Germination Of Some Corn Silage Varieties. *Journal of Tekirdag Agricultural Faculty,* 16(2): 121-132.

Dehnavi, A. R., Zahedi, M., Ludwiczak, A., Perez, S. C. and Piernik, A. (2020). Effect of salinity on seed germination and seedling development of sorghum (*Sorghum bicolor* (L.) Moench) genotypes. *Agronomy,* 10(859): 1-15.

Dornburg, V., Vashev, B., Turkenburg, W., Wicke, B., Smeets, E., Gaiser, T. and Faaij, A. (2011). The global technical and economic potential of bioenergy from salt-affected soils. *Energy & Environmental Science*, 4: 2669.

Ellis, R. H. and Roberts, E. H. (1980). Towards a Rational Basis for Testing Seed Quality. p. 605-635. In: Hebblethwaite, P.D., ed. Seed Production. Butterworths, England.

Gamborg, O. L., Miller, R. A. and Ojima K. (1968). Nutrient requirements of suspension cultures of soybean root cells. *Experimental Cell Research,* 50: 151-155.

International Seed Testing Association [ISTA] (2003). International Rules for Seed Testing. Bassersdorf, Switzerland.

Jaleel, C. A., Gopi, R., Manivannan, P., Kishorekumar, A., Sridharan, R. and Panneerselvam, R. (2007). Studies on germination, seedling vigour, lipid peroxidation and proline metabolism in Catharanthus roseus seedlings under salt stress. *South African Journal of Botany,* 73: 190-195.

Jiang, P., Cai, F., Zhao, Z.Q., Meng, Y., Gao, L.Y. and Zhao, T. H. (2018). Physiological and dry matter characteristics of spring maize in Northeast China under drought stress. *Water,* 10(1561): 1-14.

Khajeh-Hosseini, M., Powell, A. A. and Bingham, I. J. (2003). The interaction between salinity stress and seed vigour during germination of soybean seeds. *Seed Science and Technology,* 31(3): 715-725.

Maguire, J. D. (1962). Speed of germination-aid in selection and evaluation for seedling emergence and vigour. *Crop Science,* 2: 176-177.

Moss, D. N. and Hoffman, G. J. (1977). Analysis of Crop Salt Tolerance Data, pp. 258-271. In: Shain, I. and Shalhevet, J. (Eds.). Soil Salinity Under İrrigation: Process and Management. Ecological.

Munns, R., and Tester, M. (2008). Mechanisms of salinity tolerance. *Annual Review of Plant Biology,* 59: 651-681.

Nieman R. H. (1965). Expansion of bean leaves and its suppression by salinity. *Plant Physiology,* 40: 156–161.

Petrović, G., Jovičić, D., Nikolić, Z., Tamindžić, G. and Ignjatov, M. (2016). Comparative study of drought and salt stress effects on germination and seedling growth of pea. *Genetika,* 48(1): 373‑381.

Savić, J., Nikolić, R., Banjac, N., Zdravković-Korać, S., Stupar, S., Cingel, A., Ćosić, T., Raspor, M., Smigocki, A. and Ninković, S. (2019). Beneficial implications of sugar beet proteinase inhibitor BvSTI on plant architecture and salt stress tolerance in *Lotus corniculatus* L. *Journal of Plant Physiology*, 243: 1-10.

Shokat, S. and Großkinsky, D. K. (2019). Tackling salinity in sustainable agriculture—what developing countries may learn from approaches of the developed world. *Sustainability,* 11(4558): 1-19.

Snedecor, G. W. and Cochran, W. G. (1967). Statistical Methods, 6th ed. Ames, Iowa: Iowa State University Press. p 693.

Tarchoune, I., Sgherri, C., Izzo, R., Lachaal, M., Ouerghi, Z. and Navari-Izzo, F. (2010). Antioxidative responses of *Ocimum basilicum* to sodium chloride or sodium sulphate salinization. *Plant Physiology and Biochemistry,* 48: 772-777.

Teakle, N. L., Real, D. and Colmer, T. D. (2006). Growth and ion relations in response to combined salinity and waterlogging in the perennial forage legumes *Lotus corniculatus* and *Lotus tenuis*. *Plant Soil,* 289: 369-383.

Topçu-Demiroğlu, G. and Özkan, Ş. S. (2016). Effects of salt and drought stresses on germination and seedling growth of sainfoin (*Onobrychis viciifolia* Scop.). *Journal of Agricultural Faculty of Uludag University,* 30: 406-409.

Uchiya, P., Escaray, F. J., Bilenca, D., Pieckenstain, F., Ruiz, O. A. and Menendez, A. B. (2016). Salt effects on functional traits in model and in economically important Lotus species. *Plant Biology,* 18: 703-709.

Undersander, D., Mertens, D. and Thiex, N. (1993). Forage Analyses. Information Systems Division, National Agricultural Library (United States of America) NAL/USDA, 10301 Baltimore Avenue Beltsville, Md. 2070. http://www.nal.usda.gov.

Ünlüsoy, A. G., Yolcu, S., Bor, M., Özdemir, F. nad Türkan, İ. (2023). Activation of photorespiration facilitates Drought Stress Tolerance in *Lotus corniculatus*. *Journal of Plant Growth Regulation,* 42: 2088-2101.

Wang, D., Sun, Z., Hu, X., Xiong, J., Hu, L., Xu, Y., Tang, Y. and Yanmin, W. (2021). The key regulator *LcERF056* enhances salt tolerance by modulating reactive oxygen species-related genes in *Lotus corniculatus. BMC Plant Biology,* 21(605): 1-13.

Wang, L., Shi, J., Zuo, Q., Zheng, W. and Zhu, X. (2012). Optimizing parameters of salinity stress reduction function using the relationship between root-water-uptake and root nitrogen mass of winter wheat. *Agricultural Water Management,* 104: 142-152.

Wang, Y., Xu, Y., Peng, Y., Yan, J., Yan, X., Zhou, Z. and Lin, J. (2019). Cotyledon removal decreases salt tolerance during seedling establishment of Ricinus communis, an oilseed energy crop species. *Industrial Crop & Products,* 142 (2019): 1-11.

Zheng, Y., Jia, A., Ning, T., Xu, J., Li, Z. and Jiang, G. (2008). Potassium nitrate application alleviates sodium chloride stress in winter wheat cultivars differing in salt tolerance. *Journal of Plant Physiology,* 165: 1455-1465.

Zhou, M. L., Ma, J. T., Zhao, Y. M., Wei, Y. H., Tang, Y. X. and Wu, Y. M. (2012). Improvement of drought and salt tolerance in Arabidopsis and *Lotus corniculatus* by overexpression of a novel DREB transcription factor from *Populus euphratica*. *Gene,* 506: 10-17.