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Research Article

Germination Status of Birdsfoot Trefoil (*Lotus corniculatus* L.) Seeds in Different Salt Water

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Abstract

This research was carried out according to the factorial experimental design in completely randomized with 4 replications in order to determine the germination status of birdsfoot trefoil (*Lotus corniculatus* L.) seeds collected from 3 (three) locations, namely Suveren (A), Melekli (B) and Aşağı Çamurlu (C) of Iğdır, and Yalıçapkını and Maral varieties in salt water (100, 200 300 400 and 500 mM). According to different salinity water averages, birdsfoot trefoil seeds at location B showed higher normal germination than other genotypes. The abnormal germination rate of Maral variety was higher than other genotypes. There were decreases in normal germination rates of seeds due to increase in salt-water concentrations. Normal germination rate decreased to 1.7% at the highest salinity concentration (500 mM). Germination rate decreased from 78.3% to 69.6% at 100 mM level of salt water compared to control. At 200 mM level of salt water, the germination rate decreased to 23.4%. According to the research results, while there was a significant decrease in germination in other genotypes and varieties at 300 mM salinity level, a high rate of germination was observed in seeds collected in location B. Seeds collected in location B showed higher salt tolerance than seeds and varieties collected in other locations.
Keywords: *Lotus corniculatus*, seed vigor, salinity, population, variety

Farklı Tuzlu Sularda Gazal Boynuzu (*Lotus corniculatus* L.) Tohumlarının Çimlenme Durumu

Öz

Bu araştırma Iğdır'ın Suveren (A), Melekli (B) ve Aşağı Çamurlu (C) olmak üzere 3 (üç) lokasyonda toplanan gazal boynuzu tohumları ile Yalıçapkını ve Maral çeşitlerinin tuzlu suda (100, 200 300 400 ve 500 mM) çimlenme durumlarının belirlenmesi amacıyla tesadüf parsellerinde faktöriyel deneme desenine göre 4 tekerrürlü olarak yürütülmüştür. Farklı tuz su ortalamalarına göre, B lokasyonundaki gazal boynuzu tohumları diğer genotiplerden daha yüksek normal çimlenme göstermiştir. Maral çeşidinin anormal çimlenme oranı diğer genotiplerden daha yüksek olmuştur. Tuzlu su konsantrasyonlarındaki artışa bağlı olarak tohumlardaki normal çimlenme oranlarında azalmalar olmuştur. En yüksek tuzluluk konsantrasyonunda (500 mM) normal çimlenme oranı %1.7'ye kadar düşmüştür. Kontrole göre tuzlu suyun 100 mM seviyesinde çimlenme oranı %78.3'ten %69.6'ya düşmüştür. Tuzlu suyun 200 mM seviyesinde çimlenme oranı % 23.4'e düşmüştür. Araştırma sonuçlarına göre 300 mM tuzluluk seviyesinde diğer genotip ve çeşitlerde önemli oranda çimlenmede düşüş olurken, B lokasyonunda toplanan tohumlarda yüksek oranda çimlenme görülmüştür. B lokasyonunda toplanan tohumlar diğer lokasyonlarda toplanan tohum ve çeşitlerden daha yüksek tuza dayanıklılık göstermiştir.
Anahtar Kelimeler: Gazal boynuzu, çimlenme, tuzluluk, popülasyon, çeşit

Introduction

Soil salinity is a significant global issue that decreases the diversity of plant life and agricultural products worldwide. In semiarid and arid regions, poor drainage, high evaporation rates, low precipitation, agricultural practices, and specific soil conditions are the primary causes of salinity. Excessive salt in the soil deteriorates the soil structure and this reduces crop yield and quality. Salt stress causes many diseases as well as various developmental processes in plants at the morphological, cellular, physiological and molecular levels. Cultivating plants in soils that face issues such as salinity, alkalinity, and poor drainage poses significant challenges. To successfully produce plants in these areas, it's essential to either improve the soil conditions related to salinity, alkalinity, and drainage or to plant species that are resistant to these challenges.While climate change has an impact in many areas, it also causes salinity and alkalinity problems in soils. In arid and semi-arid regions, insufficient rainfall or excessive and irregular irrigation causes agricultural lands to become salinized and alkalized (Keskin and Temel, 2024). There are 954 million hectares of land affected by salinity in the world and agricultural production has been restricted in these areas (Temel ve Şimşek, 2011). Natural rangeland are rich in genetic resources and diversity due to the variety of plant species they contain. When these plant species are destroyed, it results in the loss of invaluable genetic resources as well.

Lotus species include about 200 species and are generally adapted to temperate and humid regions. *Lotus* species generally include species resistant to cold winter conditions (Beuselinck and Grant, 1995; Díaz et al., 2005). Species from the Lotus genus typically thrive in challenging conditions, as they can grow in saline, acidic, alkaline, heavy, and poorly drained soils. (Galloway et al., 2010; Azarafshan and Abbaspour, 2014; Büyükyıldız et al., 2023). The most cultivated species within the *Lotus* genus is *Lotus corniculatus*. The corniculatus is a perennial legume plant widely grown in different parts of the world. *Lotus corniculatus* is mostly cultivated in Europe, North and South America (Díaz et al., 2005). *Lotus corniculatus* is more resistant to drought conditions than many *Lotus* species (Peterson et al., 1992; Blumenthal and McGraw, 1999).

Due to its high tannin content, it does not cause bloating in animals grazing the plant, and it also has high crude protein and low fiber contents. *Lotus corniculatus* is also rich in flavan, saponin, coumarin, phenolic compounds, sterols, glycosides and mineral contents (Carter et al., 1999; Gebrehiwot et al., 2002; Uzun and Ocak, 2018; El-Gazzar et al., 2022). It is among the plants used in the improvement of rangeland (Özpınar et al., 2019; Ferat et al., 2008).

It was carried out to determine the germination capacities in different salt-water of the registered birdsfoot trefoil seeds and the seeds collected from natural areas of the plant, which has important features such as having high feed value, improving the soil and providing nitrogen to the soil, being tolerant to salinity and not causing bloat in animals. In this study, it will be seen whether the birdsfoot trefoil seeds collected in natural areas can be used alone or as a mixture in fields, meadows and pastures with salinity problems and whether these collected genotypes can be used in cultivation and breeding studies.

Materials and Methods

In the research, *Lotus corniculatus* populations collected from Suveren (A), Melekli (B) and Aşağı Çamurlu (C) locations in the center of Iğdır and the seeds of Maral and Yalıçapkını varieties of *Lotus corniculatus* provided by the Aegean Agricultural Research Institute were used.

A high rate of dormancy is observed in birdsfoot trefoil seeds collected in natural areas, and it is recommended that the best method to break this dormancy is to soak the seeds in sulfuric acid for 12 minutes (Uca, 2024). Before starting the germination experiments, birdsfoot trefoil seeds collected in natural areas were soaked in 95-98% sulfuric acid for 12 minutes and then the seeds were washed with pure water (Uca, 2024). For surface sterilization of the seeds, they were kept in 2% sodium hypochlorite for 10 minutes.

Saline waters were prepared at concentrations of 0, 100, 200, 300, 400 and 500 mM. Firstly, 58.5 g of NaCl was weighed and dissolved in 1000 ml of water to obtain 1 (one) molar salt water. To prepare salt water at different concentrations, 100, 200, 300, 400 and 500 mM water was taken from the previously prepared 1 molar salt water and 900, 800, 700, 600 and 500 ml of pure water were added, respectively, to complete the volume to 1000 ml. After placing coarse filter paper into each petri dish, 25 surface sterilized seeds were placed and 10 ml of salt water was added (Herrera and Pinto, 2009). In the first salt water application, 2% of a fungicide containing 80% thiram was added to the salt water to protect the seeds from pathogen damage. Each application was prepared in 4 (four) replications and germination status was monitored for 15 days in a cooled incubator set at 20/30 °C, where the highest germination rate was determined (Uca, 2024). When water was reduced in the petri dishes, the filter paper was changed to prevent salt accumulation, and then salt water was added. Seeds with a radicle length of 2 mm were considered normal seeds. On the last day of the germination application, seeds with a radicle length of 2 mm were counted as normal germination, seeds that did not reach 2 mm were counted as abnormal germination, and seeds that did not germinate were counted as dead seeds. Normal (N), abnormal (A) and dead seed (D) rates were determined according to the formula below (SCST, 1993).

N, A, D germination rate (%) = (seed germination status / number of seeds in the germination container) x 100

Statistical Analyses

The data obtained from the research were subjected to variance analysis according to the factorial experimental design in completely randomized with 4 replications in the JMP 5.0.1 package program. The comparison and grouping of the means that were significant as a result of variance analysis were done according to the LSD test.

Rersults and Discussion

The variance analysis table for different genotypes and salt-water applications is given in Table 1. Genotype, saline water treatments and genotype x saline water treatments interaction had significant effects on normal germination and dead seed rates. Only the effect of genotype on abnormal germination was statistically significant.

Table 1. Variance analysis results of germination in salt water

Varyasyon kaynakları	Normal (%)	Abnormal (%)	Dead seed (%)
Genotip (G)	5.65**	11.13**	3.82**
Salinity (S)	355.9**	1.48 ns	328.9**
G x S int.	10.82**	1.02 ns	9.74**

**: Significant at 1% level, ns: not significant,

According to the average of saline water applications, the normal germination rate of seeds belonging to the genotypes varied between 27.2% and 37.9%, the abnormal germination rate varied between 3.2% and 7.5% and the dead seed rate varied between 57.4% and 65.4%. Birdsfoot trefoil genotypes collected at location B showed higher normal germination and lower dead seed ratio than other genotypes. Abnormal germination rate was high in Maral variety. Increased salt concentration in water caused a decrease in normal germination rate and an increase in the rate of dead seeds. (Table 2). Plant species and varieties show different levels of resistance to salinity (Teakle et al., 2006; Galloway et al., 2010; Azarafshan and Abbaspour, 2014; Hajri et al., 2018; Keskin et al., 2023). This situation is due to the genetic characteristics of the plants.

Table 2. Germination rates in different salt water applications (%)

Genotip	Normal (%)	Abnormal (%)	Dead seed (%)
A	31.8 b	4.0 cd	64.2 a
B	37.9 a	4.7 bc	57.4 b
C	31.0 bc	5.5 b	63.5 a
Yalıçapkını	31.8 b	3.2 d	65.0 a
Maral	27.2 c	7.5 a	65.4 a
Tuzluluk (T)			
Control	78.3 a	4.0	17.7 e
100 mM	69.6 b	5.0	25.4 d
200 mM	23.4 c	4.8	71.8 c
300 mM	14.6 d	4.6	80.8 b
400 mM	4.1 e	5.8	90.1 a
500 mM	1.7 e	5.6	92.7 a

There is no statistically significant difference between numbers indicated by the same letters.

The normal germination rates of birdsfoot trefoil seeds at A location were significantly affected in 100 mM saline water. The normal germination rates of birdsfoot trefoil seeds at location B started to decrease after 300 mM saline water level. On the other hand, the normal germination rates of seeds at location C and seeds of Yalıçapkını and Maral varieties decreased significantly after 100 mM salt-water application. Birdsfoot trefoil genotypes responded differently to germination in salt water (Figure 1).

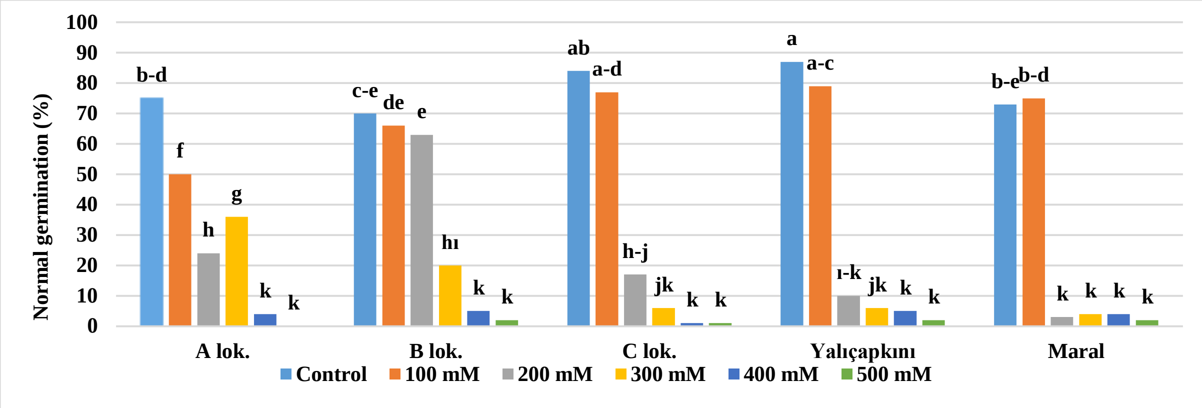

Figure 1. Normal germination rates in genotype and saline water treatment (%)

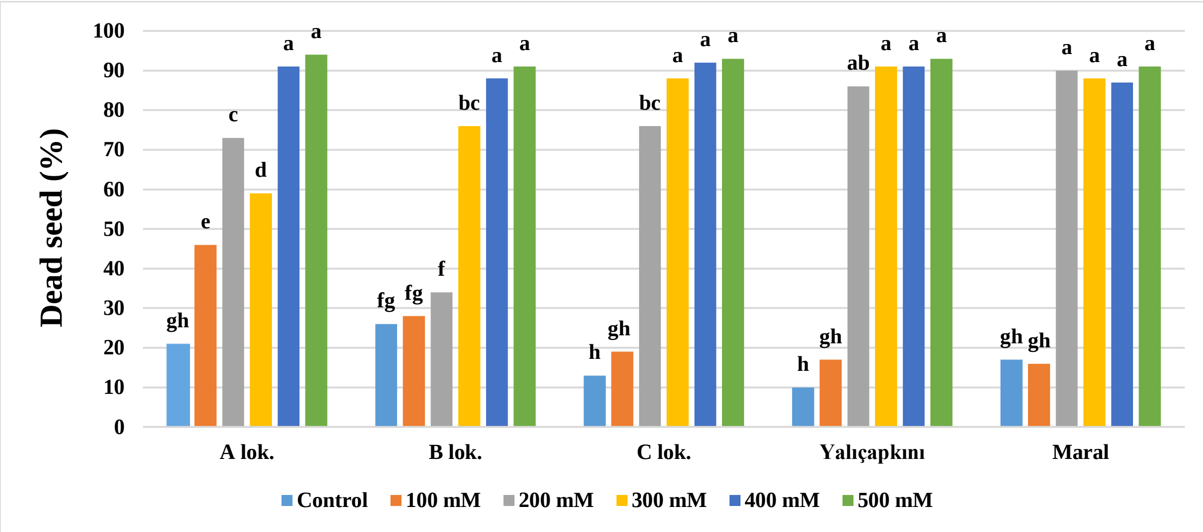

Figure 2. Dead seed rates in genotype and saline water treatment (%)

The normal germination rates of birdsfoot trefoil seeds at location A were significantly affected in 100 mM saline water. The normal germination rates of birdsfoot trefoil seeds at location B started to decrease after 300 mM saline water level. On the other hand, the normal germination rates of seeds at location C and seeds of Yalıçapkını and Maral varieties decreased significantly after 100 mM salt-water application. Birdsfoot trefoil genotypes responded differently to germination in salt water (Figure 1).

The highest dead seed ratio was obtained in 400 mM and 500 mM saline water applications to seeds in locations A and B, while 300 mM, 400 mM and 500 mM saline water applications to seeds in location C were obtained. On the other hand, the highest dead seed rates in Yalıçapkını and Maral varieties were obtained in 200 mM, 300 mM, 400 mM and 500 mM salt water applications. (Figure 2).

Birdsfoot trefoil is resistant to salty soils and is among the plants that can be grown in salty soils (Uzun et al., 2008). Birdsfoot trefoil can sustain plant development up to 400 mM salinity level (Teakle et al., 2006). The study conducted by Valiente et al. (2007) determined that there was no significant decrease in seed germination in 200 mM saline water application of *Lotus creticus* seeds compared to the control. In the study conducted by Galloway et al. (2010), it was reported that *Lotus corniculatus*, *Lotus tenuis* and *Lotus pedunculatus* species gave different responses to saline water application. They reported that there was no significant decrease in yield up to 150 mM saline water level. Azarafshan and Abbaspour (2014) reported that different varieties of *Lotus corniculatus* responded differently to saline water treatment and plant growth was significantly affected with increasing saline water treatment. It has been reported that the salt tolerance of *Lotus creticus* and *Lotus ornithopodioides* seeds collected in natural environments is different and that they are resistant to saline water levels up to 150 mM and 250 mM depending on the locations (Hajri et al., 2018). As seen in previous studies, it has been reported that species of the *Lotus* genus and natural populations and varieties of the *Lotus corniculatus* species respond differently to salt water application. This shows that there is a great diversity in the genetic structure of birdsfoot trefoil developing in different natural areas.

Conclusion

According to the research results, there were decreases in the normal germination rate of seeds due to the increase in the amount of salt in the water. At the highest salinity level (500 mM), 1.7% of the seeds had normal germination. There were significant decreases in normal germination rates in seeds at location A immediately after control application and in seeds at location C and in seeds of Yalıçapkını and Maral varieties after 100 mM saline water level. On the other hand, in the seeds at location B, significant decreases in normal germination began to be observed after the 300 mM saline water level. Compared to other genotypes and varieties, seeds collected in location B in high saline water showed higher germination. By setting up trials in pots and fields with saline environments, the seedling and plant development status of this plant can be investigated and its usage status can be revealed and it can also be used in breeding studies.

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Authors' Contributions

Authors declare that they have contributed equally to the article.

Conflicts of Interest Statement

The authors declare that there is no conflict of interest.

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