

The Effect of Pre-Germination Treatment with L-Proline on the Germination Dynamics of Forage Soybean Seeds Under Salt Stress

Tuz Stresi Altındaki Yemlik Soya Fasulyesi Tohumlarının Çimlenme Dinamikleri Üzerine L-Prolin ile Çimlenme Öncesi İşlemin Etkisi

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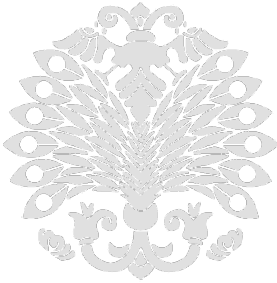


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ABSTRACT

The present study was conducted with the objective of evaluating the effects of pre-sowing L-proline (LP) applications on the germination dynamics of forage soybean seeds under salt stress conditions. The seeds used in the study were soaked in LP solutions at concentrations of 0, 50, 100, and 150 mg L⁻¹ for 24 hours at 15°C. Germination tests were then conducted at NaCl concentrations of 0, 50, 100, and 150 mM. The study was conducted under controlled conditions in accordance with the principles established by ISTA. The experiment was conducted in four replications. The study evaluated the following parameters: total germinated seeds (TGS), germination rate (GR), germination speed index (GSI), average germination time (AGT), germination rate coefficient (GRC), and sensitivity index (SI). The results showed that LP treatments alone had no significant effect on the germination parameters examined. In contrast, it was found that increasing NaCl concentrations significantly reduced GSI and GRC values, while prolonging AGT. The sensitivity index increased with increasing salt concentrations, and the LP x NaCl interaction was found to be significant for this parameter. The highest SI value (1.504) was observed in the combination of 150 mg L⁻¹ LP and 150 mM NaCl. This indicates that high LP doses may exacerbate rather than alleviate stress effects under severe salinity conditions. In general, while LP had no significant effect on overall germination rates, increasing NaCl concentrations severely disrupted germination dynamics. It has been concluded that the effects of amino acid applications depend largely on dosage, stress levels, and their interactions. These findings underscore the importance of optimizing germination conditions and indicate the need to investigate alternative amino acid types, application methods, and dosages to enhance the stress tolerance of forage soybean plants during the germination period.

Keywords: Abiotic stress, amino acid, forage soybean, germination, NaCl

ÖZ

Soya üretimi çimlenme ve erken fide gelişimi aşamalarında tuz stresi nedeniyle sıklıkla kısıtlanmaktadır. Bu çalışma, tuz stresi koşulları altında yemlik soya fasulyesi tohumlarının çimlenme dinamikleri üzerine ekim öncesi L-prolin (LP) uygulamalarının etkilerini değerlendirmek amacıyla gerçekleştirilmiştir. Çalışmada kullanılan tohumlar, 15°C'de 24 saat boyunca 0, 50, 100 ve 150 mg L⁻¹ konsantrasyonlarında LP çözeltilerinde iletılmıştir. Ardından, 0, 50, 100 ve 150 mM NaCl konsantrasyonlarında çimlenme testleri yapılmıştır. Çalışma, ISTA tarafından belirlenen ilkelere uygun olarak kontrollü koşullar altında gerçekleştirilmiştir. Deney dört tekerrürlü olarak gerçekleştirilmiştir. Çalışmada; toplam çimlenen tohum sayısı (ÇTS), çimlenme oranı (ÇÖ), çimlenme hızı indeksi (ÇHI), ortalama çimlenme süresi (OÇT), çimlenme oranı katsayısı (ÇOK) ve duyarlılık indeksi (DI) parametreleri değerlendirilmiştir. Sonuçlar, LP uygulamalarının tek başına incelenen çimlenme parametrelerini olumsuz etkilemiştir.

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Buna karşılık, artan NaCl konsantrasyonlarının ÇHI ve ÇOK değerlerini önemli ölçüde düşürdüğü, ÇS süresini ise uzattığı saptanmıştır. DI, tuz konsantrasyonlarının artmasıyla birlikte yükselmiş ve bu parametre açısından LP ile NaCl arasındaki etkileşimin anlamlı olduğu görülmüştür. En yüksek SI değeri (1,504), 150 mg L⁻¹ LP ve 150 mM NaCl kombinasyonunda gözlemlenmiştir. Bu, yüksek LP dozlarının şiddetli tuzluluk koşulları altında stres etkilerini hafifletmek yerine daha da şiddetlendirebileceğini göstermektedir. Genel olarak, LP'nin genel çimlenme oranları üzerinde önemli bir etkisi olmamasına rağmen, artan NaCl konsantrasyonları çimlenme dinamiklerini ciddi şekilde bozmuştur. Amino asit uygulamalarının etkilerinin büyük ölçüde doza, stres seviyelerine ve bunların etkileşimlerine bağlı olduğu sonucuna varılmıştır. Bu bulgular, çimlenme koşullarının optimize edilmesinin önemini vurgulamakta ve çimlenme döneminde yemlik soya fasulyesi bitkilerinin stres toleransını artırmak için alternatif amino asit türleri, uygulama yöntemleri ve dozajlarının araştırılması gerektiğini göstermektedir.

Anahtar Kelimeler: Abiyotik stres, amino asit, yemlik soya fasulyesi, çimlenme, NaCl

Introduction

Adequate and balanced nutrition stands out as one of the primary challenges facing the modern world, and one that is growing in importance. For this reason, establishing a socially, economically, and environmentally sustainable agricultural sector and ensuring that the public has access to high-quality and sufficient nutrition are among the fundamental objectives of agricultural policies. In achieving these goals, increasing livestock production plays a vital role, and the sustainability of such production depends largely on forage crop production (Ministry of Agriculture and Forestry of the Republic of Turkey, 2025).

Forage crops and pastures are among the primary sources for both plant-based products consumed directly in human diets and the production of red meat and other animal products. These resources stand out as high-quality roughage due to their low cost and suitability for the digestive physiology of ruminants (Serin and Tan, 2001). Pastures and rangelands, which are the primary sources of these roughages, along with forage crops that can be incorporated into crop rotations, play a significant role in increasing animal production and developing sustainable agricultural systems (Gökkuş, 2011).

Forage crops reduce production costs and support high-quality animal production by providing nutritious roughage (Lebacqz et al., 2015; Lee, 2018). They also improve production stability through continuous forage supply (Cosentino et al., 2014). In addition, forage crops enhance soil fertility, reduce erosion, improve soil moisture and structure, and increase organic matter and microbial activity (Fullen, 1998; Rasmussen et al., 2012; Triberti et al., 2016). They further contribute to greenhouse gas balance, biological pest control, and agroecosystem biodiversity (Liu et al., 2022; Meiss et al., 2010; Shackelford et al., 2013). Despite these benefits, especially legume forage crops are

affected by various biotic and abiotic stress factors.

Adverse environmental conditions encountered during the germination and emergence stages can cause significant losses in the early stages of legume forage crop development. Stress factors such as drought, low temperatures, and salinity both reduce seed germination rates and cause uneven emergence. This situation, in turn, has negative effects on yield and quality during the later stages of plant development. In conclusion, methodologies that enhance the efficiency of developmental processes during the early stages of crop cultivation are of great importance for forage crop production (Mishra et al., 2025).

Forage crops are divided into two categories: grasses, which are used as a source of energy, and legumes, which are a rich source of protein. Legumes, in particular, are of significant importance due to their high protein content and their ability to fix nitrogen biologically (Islam et al., 2022; Shah et al., 2021). Indeed, these forage crops make a significant contribution to livestock farming by providing large quantities of high-quality roughage and enhancing soil fertility. Soybeans, a legume forage crop, are recognized worldwide as a strategic crop due to their high protein content (35–45%) and wide range of applications.

Soybeans are a significant crop that has the potential for use in both the production of human food and animal feed (Dei, 2011; Sharma et al., 2014). Nevertheless, soybean cultivation is notably vulnerable to diverse environmental stress factors, particularly during the processes of germination and early seedling development. Among these problems, salinity stress stands out; it delays seed germination, reduces germination rates, and negatively affects seedling development, thereby significantly reducing soybean yield (Kan et al., 2015; Zhang et al., 2016).

One of the methods developed to mitigate the adverse effects caused by salinity and similar abiotic stresses is pre-

germination (Anghla et al., 2025). Pre-germination is based on the principle of treating seeds under controlled conditions using hydro-priming, hormone priming, osmo-priming, and bio-priming techniques to metabolically initiate the germination process (Khan et al., 2022). Thanks to this application, seed germination rates increase, emergence becomes more uniform, and plants' tolerance to stressful conditions improves (Jatana et al., 2024).

Among these methods, amino acid-based applications have gained prominence in recent years due to their potential to enhance plant resilience, particularly under abiotic stress conditions. In this context, proline is a key amino acid whose accumulation increases under environmental factors such as salinity, drought, heavy metal stress, low temperatures, and UV radiation (Bassi and Sharma, 1993; Hare et al., 1998; Munns, 2005; Naidu et al., 1991; Rhodes et al., 2002; Sharma and Dietz, 2006). Proline plays a role in osmotic regulation, maintaining cellular redox balance, membrane stability, and the detoxification of reactive oxygen species (ROS). Proline also functions as an antioxidant and signaling molecule, contributing to stress signaling and cellular renewal processes. Additionally, it plays a role in physiological processes such as flowering, pollen development, leaf growth, and embryo formation. It has been reported that proline accumulation under salt stress is positively correlated with stress tolerance and is found at higher levels in tolerant plants (Ashraf and Foolad, 2007; Fougere et al., 1991; Hosseinifard et al., 2022; Petrusa and Winicov, 1997). In addition, it has been reported that proline can promote seed germination under abiotic stress conditions such as drought and salinity (Guo et al., 2022; Shahid et al., 2022).

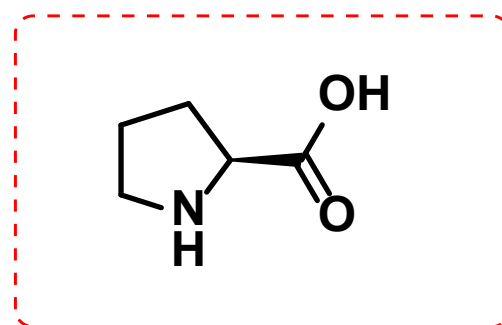
This study evaluated the germination performance of forage soybean seeds at different salt concentrations following a pre-treatment with L-proline. The aim of the study was to determine the effects of L-proline treatment on germination performance under salt stress conditions and to highlight the treatment's potential for sustainable agricultural production.

Methods

In the study, seeds of the Yemsoy variety of forage soybean (*Glycine max* L.) were used as the plant material. Seed pre-germination treatments were conducted in a climate-controlled chamber at the laboratory of the Faculty of Agriculture, Atatürk University; the seeds were treated with 0, 50, 100, and 150 mg L⁻¹ L-proline (LP) solutions, and subsequently exposed to 0, 50, 100, and 150 mM NaCl salt concentrations, followed by the procedures detailed below.

Figure 1

The Molecular Structure of the L-Proline Compound



L- Prolin

Pre-Germination Treatments

The seeds were soaked in solutions containing 0 (control), 50, 100, and 150 mg L⁻¹ L-proline in distilled water, and then subjected to pre-germination for 24 hours at 15°C in a climate chamber. For each treatment, 4 g of forage soybean seeds were subjected to pre-germination in Petri dishes containing 8 mL of treatment solution (1:2 seed:solution ratio). After treatment, the seeds were spread on filter paper and dried at room temperature until they reached their pre-treatment moisture content. In the study, control seeds and seeds treated with L-Proline were subjected to germination and emergence tests. The number of germinated seeds was counted daily for all treatments. The number of germinated seeds was counted daily, and the germination rate was determined as a percentage.

Standard Germination Test

To determine the effect of pre-germination treatments on germination performance under NaCl stress, forage soybean seeds were subjected to germination in 0, 50, 100, and 150 mM NaCl solutions. To determine the germination rates before and after pre-germination, seeds were placed in petri dishes in accordance with International Seed Testing Association (ISTA) guidelines, with each treatment consisting of 4 replicates and 25 seeds per replicate and subjected to a germination test in an incubator at 20°C for 10 days (ISTA, 2009). Four milliliters of salt (NaCl) solution were added to each petri dish. When moisture levels in the germination medium decreased, fresh salt solutions were added to the medium. Daily counts were conducted, and seeds with rootlets ≥ 2 mm were considered germinated. In the study, the parameters total germinated seeds (TCT), germination rate (CO), germination rate index (CHI), sensitivity index (HI), average germination time (OCS), and

germination rate coefficient (CHK) were calculated using the following formulas (Elkoca, 1997; Ellis and Roberts, 1980; Maguire, 1962; Pedersen et al., 1993; Yıldırım and Güvenç, 2006).

$$GR (\%) = \left(\frac{\text{Total number of germinated seeds}}{20} \right) \times 100$$

$$AGT (\text{gün}) = \frac{\Sigma(g \times n)}{Sn}$$

$$GRI = n_1/t_1 + n_2/t_2 + n_3/t_3 + \dots + n_n/t_n$$

$$GRC = \frac{\Sigma f \times x}{\Sigma f}$$

$$SI = \frac{OCZ(\text{tuz})}{OCZ(\text{kontrol})}$$

Results

The results of the analysis of variance (ANOVA) regarding the effects of different L-proline (LP) and salt (NaCl) doses on the germination and emergence characteristics of forage soybean seeds are presented in Table 1. According to the results, the effects of LP dose, NaCl dose, and the LP x NaCl interaction on total germinated seeds (TGS) and germination rate (GR) were not found to be statistically significant. In contrast, the effect of NaCl doses on the germination rate index (GRI) and germination rate coefficient (GRC) was found to be significant at the $p < .01$ level, while the effect on the average germination time (AGT) was significant at the $p < .05$ level. Regarding the sensitivity index (SI), the LP dose, NaCl dose, and LP x NaCl interaction were found to be significant at the $p < .01$ level (Table 1).

Table 1

Results of The Analysis of Variance for TGS, GR, GRI, SI, AGT, and GRC Data Following Different L-Proline (LP) and Salt (NaCl) Treatments

*: $p < .01$, **: $p < .05$

SV	DF	TGS	GR	GRI	SI	AGT	GRC
Block	2	2.63	2.63	1.96	0.57	2.50	2.70
LP	3	0.15	0.15	0.16	4.90e+27	0.40	0.55
					**		
NaCl	3	2.88	2.88	10.05	5.91e+28	4.78	10.88
				**	**	**	**
LP x NaCl	9	0.61	0.61	1.26	4.65e+27	0.38	1.16
					**		
Hata	30	-	-	-	-	-	-

When the average effects of LP doses were examined, no statistically significant differences were observed between treatments in terms of TGS, GR, GRI, SI, AGT and GRC values (Table 2). Depending on the LP dose, TGS values ranged from 12.50 to 13.25, GR values from 50.00% to 53.00%, GRI values ranged from 5.08 to 5.48, SI values from 1.103 to 1.202, AGT values from 2.53 to 2.76 days and GRC values from 30.49 to 32.79. These results indicate that the applied amino acid doses did not have a significant main effect on the germination characteristics examined at the general average level.

Table 2

Effects of Different L-Proline (LP) and Salt (NaCl) Doses on TCT, CO, CHI, HI, OCS, And CHK Values

Factor	Dose	TGS	GR (%)	GRI	SI	AGT	GRC
LP	0	12.92	51.67	5.33	1.180	2.71	31.86
				a	a	a	a
	50	13.25	53.00	5.48	1.166	2.68	30.98
				a	a	a	a
	100	12.83	51.33	5.35	1.103	2.53	32.79
NaCl				a	a	a	a
	150	12.50	50.00	5.08	1.202	2.76	30.49
				a	a	a	a
	0	13.75	55.00	6.70	0.978	2.25	37.62
				a	c	b	a
NaCl	50	14.25	57.00	6.06	1.159	2.66	31.51
				a	b	ab	b
	100	12.17	48.67	4.76	1.174	2.70	30.11
				b	b	ab	b
	150	11.33	45.33	3.72	1.340	3.08	26.88
				b	a	a	b

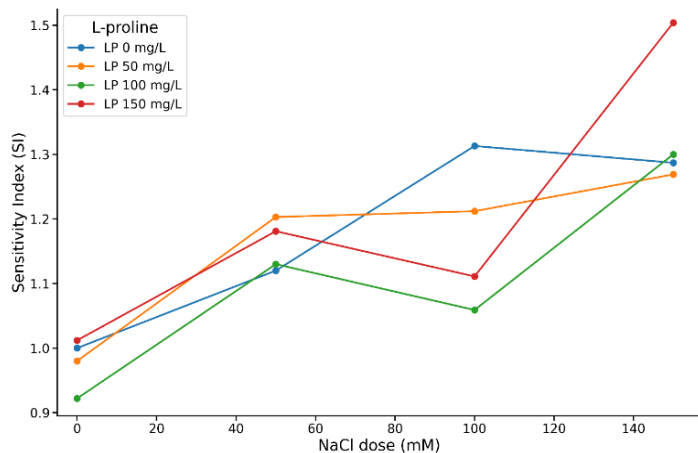
*There is no statistically significant difference between the values denoted by the same letter.

When the average effects of NaCl concentrations were evaluated, no significant difference was observed between concentrations in terms of TGS and GR. In contrast, it was found that NaCl doses had a significant effect on GRI, SI, AGT and GRC (Table 2). The highest GRI value was obtained at the 0 mM NaCl dose (6.70), followed by the 50 mM dose (6.06). The lowest GRI value was determined at the 150 mM NaCl dose (3.72). Similarly, the GRC value decreased with increasing salt dose, the highest value was observed at the 0 mM dose (37.62), and the lowest value at the 150 mM dose (26.88). In contrast, the AGT value increased with rising NaCl levels; the lowest value was observed at the 0 mM dose (2.25 days), and the highest value at the 150 mM dose (3.08 days). These findings indicate that high salt concentrations slow down the germination process and negatively affect germination performance (Table 2).

When evaluating the mean values of the main effects in terms of SI, it was observed that the sensitivity index increased with rising NaCl concentrations. The lowest SI value was obtained at a 0 mM NaCl dose (0.978), while the highest value was observed at a 150 mM NaCl dose (1.340). However, since the LP x NaCl interaction was significant for the SI trait, a detailed evaluation of this trait should be conducted using the interaction means.

The effects of the LP x NaCl interaction on SI are presented in Figure 1. According to the data, the highest SI value (1.504) was observed in the 150 mg L⁻¹ LP + 150 mM NaCl combination, while the lowest value (0.922) was observed in the 100 mg L⁻¹ LP + 0 mM NaCl combination. A general trend of increasing SI values was observed as the salt concentration increased, however, the extent of this increase varied depending on the amino acid concentration. Notably, at a 150 mg L⁻¹ LP dose, the SI value rose more markedly at high salt levels compared to other combinations. This indicates that amino acid application can modulate the sensitivity index under salt stress conditions depending on the dose level (Figure 2).

Figure 2
Interaction Effect of Different L-Proline (LP) and Salt (NaCl) Treatments on The Sensitivity Index (SI)



Overall, no significant main effect of amino acid treatments alone was observed on the germination characteristics examined; in contrast, salt stress negatively affected parameters related to germination rate in particular. With regard to the sensitivity index, it was concluded that both amino acid dose and salt level are important, and that evaluating this characteristic on a combined basis is more appropriate.

Discussion

This study evaluated the effects of different doses of LP and

NaCl on the germination characteristics of forage soybean seeds. According to the results, LP applications alone had no significant effect on the parameters examined. However, salt stress has had a significant negative impact, particularly on parameters such as AGT, GR, and SI. Indeed, the findings from this study are consistent with previous research indicating that the effect of LP applications may vary depending on the type, application rate, and intensity of the stress (Ashaf and Food, 2007; Badran et al., 2015; Wang and Han, 2009).

The fact that LP and salt concentrations did not result in significant differences in GR and TGS values indicates that the germination capacity of forage soybean seeds is sensitive to a certain level of salinity. In this context, it appears that salt stress conditions have no adverse effect on the germination rate, whereas the germination rate is sensitive to these stress conditions. The findings are consistent with previous studies conducted on this topic.

Indeed, the study found that the GRC and GRI values decreased significantly with increasing NaCl concentrations. The reduction in germination rate due to salt stress can be attributed to delayed water uptake caused by osmotic stress and ion toxicity, slowed metabolic processes, increased production of reactive oxygen species, and cellular damage, all of which delay the germination process (Farissi et al., 2011; Meena et al., 2019). Similarly, the increase in AGT indicates that the germination process of the seeds has been prolonged and that physiological processes are delayed under stress conditions. The findings indicate a clear negative correlation between high salt concentrations and germination dynamics.

The fact that LP applications alone do not produce a significant effect indicates that exogenous proline is not a germination-promoting factor under all conditions. Studies have shown that proline acts as an important osmoregulatory, protects proteins, maintains membrane stability, and plays a role in ROS detoxification (Meena et al., 2019). However, it is noted that the effect of exogenous proline administration may vary depending on the method of administration, the type of tissue, the dose, and the severity of stress (Hosseinifard et al., 2022). Indeed, some studies have reported that the application of proline at appropriate doses increases the germination rate. It has been found that the application of 10 mM proline to black mulberry seeds raised the germination rate to over 90% (Kaçal et al., 2020). However, it is known that this effect varies depending on both the species and environmental conditions. Therefore, the fact that the doses or application conditions used in the study were not optimal may have

prevented the expected beneficial effects of the amino acids from being observed.

The finding that the applied factors and their interactions were statistically significant suggests that this parameter functions as a more sensitive indicator of environmental stresses. The increase in the SI value with rising salt concentrations indicates that the plant is more sensitive to stress conditions. The fact that the SI reaches its maximum value under high LP (150 mg L⁻¹) and NaCl (150 mM) conditions suggests that high-dose LP application does not always have a stress-reducing effect and may potentially exacerbate stress-induced effects under certain conditions. This also indicates that amino acids such as proline, which function as osmoregulatory agents, provide benefits only when applied at appropriate doses and may disrupt metabolic balance at high doses. Indeed, the findings recorded in the present study are consistent with those obtained in numerous studies (Ehsanpour and Fatahian, 2003; Jain et al., 2001; Meena et al., 2019).

The results of the studies indicate that the effect of LP applications on germination is limited and that this effect may vary depending on the application rate, method, and plant species. However, it appears that salt stress has significant and adverse effects, particularly on germination rate and duration.

Conclusion and Recommendations

This study was conducted to determine the effect of different LP and salt concentrations on the germination characteristics of forage soybean seeds. The data obtained showed that LP application alone did not have a statistically significant effect on total germination or the germination rate. Furthermore, it was observed that salt stress did not have significant negative effects on parameters related to the germination rate. It was determined that increases in NaCl concentrations led to a significant decrease in GRC and GRI values, concurrent with an increase in TGS. Additionally, the rise in SI values with high NaCl doses clearly demonstrated the negative effect of salinity on plant vitality. The statistically significant effect of the LP x NaCl interaction, particularly on SI, indicates that LP applications have effects dependent on environmental stress conditions. Considering that high LP doses increase SI values at high salt concentrations, it was concluded that these applications do not have a stress-reducing effect under all conditions.

In conclusion, determining the appropriate concentration and application conditions for LP treatments aimed at improving germination performance in forage soybeans is of great importance. Additionally, it is acknowledged that the adverse effects of salt stress function as more sensitive indicators. Subsequent studies should examine various amino acid types, dose combinations, and application methods in detail.

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