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EFFECTS OF SALICYLIC ACID SEED PRIMING ON GERMINATION OF LENTIL (*Lens culinaris* Medik.) EXPOSED TO SALT STRESS

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Abstract: Lentil (*Lens culinaris* Medik.) is an essential crop globally, particularly in Türkiye, West Asia, Southern Europe, India, and Africa. Lentil is a valuable food source, rich in proteins, carbohydrates, minerals, and vitamins. However, lentil production faces challenges due to salinity stress, which hampers water uptake and causes toxic effects on plants. The study aimed to investigate the effects of salicylic acid (SA) seed priming and varying salt (NaCl) concentrations on the germination and development of lentil seeds. The research was conducted in the Field Crops Biotechnology Laboratory, University of Dicle, Faculty of Agriculture, Türkiye. In the research, control, two salt (NaCl), four salicylic acid (SA) doses and their combinations were used on *Lens culinaris* variety called İlke. Germination percentage, energy, rate index, mean germination time, peak value, and vigor index were calculated. The results demonstrated that SA had a significant impact on improving lentil germination under salt stress conditions. Specifically, lower concentrations of NaCl combined with SA showed positive effects on germination and growth parameters compared to higher NaCl concentrations. The highest germination percentage, energy, and rate index values were in the control and 0.25 SA dose treatment. Salicylic acid doses under 50 NaCl concentration exhibited higher germination percentage, energy, and rate index values than under 100 NaCl. Increasing SA and salt doses negatively affected lentil seed germination. This finding is significant for lentil cultivation in salt-affected areas, offering a potential strategy to improve crop resilience and yield in challenging environmental conditions.

Keywords: Lentil, *Lens culinaris* Medik., Salt stress, Salicylic acid, Germination

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1. Introduction

Lentil is a legume crop with 2n=14 chromosomes and an annual growth cycle. It is an important crop in Türkiye, following peas and chickpeas (Ladizinsky, 1979; Jha and Halder, 2016; Sehgal et al., 2021). In Türkiye, lentils are produced on 81,741 hectares of land, yielding 353,631 tons of product (FAO, 2019). Lentils are also cultivated in West Asia, Southern Europe, India, and Africa.

Lentils are a nutritious food source high in carbohydrates, protein, minerals, and vitamins (Migliozzi et al., 2015). The protein content varies depending on growing conditions, techniques, and varietal characteristics, but it averages 22.7-31.9% (Toklu et al., 2009). Lentils are essential for human and animal nutrition, especially for those who consume vegan and vegetarian products.

Plants are exposed to biotic and abiotic stress factors (Georgieva and Vassileva, 2023). Biotic stress conditions refer to the impact of bacteria, fungi, plants, and animals, while abiotic stress conditions include radiation, drought, salinity, and variations in temperature and light intensity. Reactive oxygen species (ROS) are produced and accumulated by plants under different stress conditions (Guo et al., 2023). Disruptions in metabolic events lead to oxidative stress, which causes damage to DNA, enzyme inactivation, and lipid peroxidation (Tounekti et al., 2013).

Legumes are highly sensitive to salinity stress, which can reduce yield (Farooq et al., 2017). Salinity stress can reduce plant quality and yield and negatively affect soil structure. Salinity can increase due to evaporation at high temperatures and uncontrolled irrigation. Physiological drought is caused by salinity stress, which prevents water uptake from plant roots and can have a toxic effect on glycophytic plants due to the accumulation of salt ions (Tester and Davenport, 2003). Salinity stress can reduce the germination rate and cause yield losses by preventing germination (Ondrasek et al., 2022). According to AL-Tawaha et al. (2013), salinity hurts the growth and development of lentils. Munns (2002) found that plant cells lose water and shrink in volume shortly after salt is applied.

Several growth regulators have been shown in numerous studies to effectively prevent salt stress. Studies, including the use of salicylic acid, a phenolic compound that belongs to the hydroxyl group. Salicylic acid helps prevent oxidative damage caused by water stress from salt accumulation in plant roots and protects protein structure (Kaydan et al., 2007; Vicente and Plasencia, 2011). Choudhary et al. (2021), Idrees et al. (2011) and Lee et al. (2010) found that applying salicylic acid increased germination rates by preventing salt stress. Jain and Srivastava (1981) and Ramanujam et al. (1998) reported that salicylic acid, when applied at low concentrations, accelerates vegetative growth, and increases nodule formation, flowering, and pod number, particularly in legumes, resulting in a positive effect on grain yield. Additionally, Senaratna et al. (2000) and Kaydan and Yagmur (2006) found that salicylic acid applications in beans and tomatoes increase plant tolerance to drought and frost stress.

There is study aimed was to investigate the effects of different salicylic acid seed priming and salt concentrations on the germination and development of lentil.

2. Materials and Methods

The research was conducted in the Field Crops Biotechnology Laboratory, Faculty of Agriculture, Dicle University.

In the research, control, 2 salt (NaCl), 4 salicylic acid (SA) doses and their combinations were used on *Lens culinaris* variety called İlke (Table 1).

Table 1. Salt (NaCl), Salicylic acid (SA) and NaCl + SA combinations

Treatment	Combinations	
Control	50 NaCI +	0.25
NaCI (mM /L)		0.50
50		0.75
100		1.0
SA (mM/L)	100 NaCI +	0.25
0.25		0.50
0.50		0.75
0,75		1.0
1.0		

SA= salicylic acid, NaCl= salt, NaCl + SA= salt + salicylic acid.

The experiment followed the ISTA (1996) rules. To ensure surface sterilization of seeds, they were shaken in a 5% sodium hypochlorite solution for three minutes and then washed with distilled water. Excess water from the surface-sterilized seeds was removed with sterile filter papers. The experiment was arranged in randomized plots with three replications, using 40 lentil seeds for each treatment.

For salicylic acid pretreatment, seeds were soaked for 24 h in solutions of different concentrations (0.25, 0.50, 0.75, 1.0 mM/L). For the salt treatment, irrigation water with different salt concentrations (50 and 100 mM NaCl/L) was used. The seeds were soaked in distilled water for the control treatment. The Petri dishes were then incubated in a germination cabinet at 25±2 °C (Doruk Kahraman and Topal, 2024). The germination paper was changed every two days for the duration of the experiment. Germinating seeds were defined as those with a length of the radicle of more than 2 mm (Doruk Kahraman and Topal, 2024). Measurements of the radicle and shoot traits were made after the seeds had been transferred to containers of 20 x 5 x 10 cm on the fourth day. Radicle and shoot lengths were measured with a ruler in cm, and radicle and shoot fresh weights were measured with a precision balance with an accuracy of 0.0001 g.

Germination percentage (GP) (%) (Equation 1), germination energy (GE) (%) (Equation 2), germination rate index (GRI) (Equation 3), mean germination time (MGT) (Equation 4), peak value (PV) (Equation 5), and vigour index (VI) (Equation 6) values were calculated using the following formulas:

$$GP = \frac{Number\ of\ seeds\ germinated}{Total\ number\ of\ seeds\ tested} \times 100 \tag{1}$$

$$GE = \frac{No. of\ seeds\ germinatied\ on\ day\ (4.day)}{Total\ number\ of\ seeds\ tested} \times 100 \tag{2}$$

$$GRI = \left(\frac{G1}{1}\right) + \left(\frac{G2}{2}\right) + \left(\frac{G3}{3}\right) + \cdots \left(\frac{G\dot{I}}{\dot{I}}\right) \tag{3}$$

$$MGT = \frac{\sum Fx}{\sum (F * x +, \ldots)} \tag{4}$$

$$PV = \frac{\text{Highest seed germinated}}{Number\ of\ days} \tag{5}$$

$$VI = (Average\ root\ length + Average\ hypocotle\ length) * GP \tag{6}$$

2.1. Statistical Analysis

The data obtained from the study were subjected to ANOVA analysis in the JMP 14 PRO statistical program. Differences between means were determined according to 0.05 in the Duncan test.

3. Results and Discussion

The effects of NaCl, salicylic acid and their combinations on *Lens culinaris* Medik (İlke cv.) germinated seeds were observed during for seven days (Figure 1). In the control group, 17 out of 40 seeds were recorded as germinated on the first day, and the germination of 36 seeds was completed on the 3rd day of the experiment, and 4 seeds weren't germinated during the experiment. The highest number of germinated seeds on the first day was observed at 0.25 SA dose and 0.50 SA dose. Under 50 NaCl concentration, 5 germinated seeds were recorded on the first day, and the highest number of germinated seeds was reached on the 5th day with 13.7 seeds. Under 100 NaCl concentration, seed germination occurred on the first day, but no germinated seeds were observed on the following days. SA treatment doses at 100 NaCl concentration regressed germination compared to the 50 NaCl concentration and significantly reduced the number of germinating seeds.

The effects of NaCl, salicylic acid and their combinations on the germination percentage, germination energy and germination rate index of *Lens culinaris* Medik (İlke cv.) seeds were significant (Table 2, Figure 2).

The highest germination percentage, energy and rate index values were in the control (90.0%, 90.00% and 76.53, respectively). 100 NaCl concentration was exhibited the lowest germination percentage, energy and rate index values (3.33%, 2.50% and 1.32%, respectively). The values for germination percentage, energy and rate index under 50 NaCl concentration were 34.2%, 30.0% and 22.8%, respectively. 50 NaCl concentration showed lower germination values than the control, SA, and salt + SA combinations, although not as much as 100 NaCl.

The values for germination percentage, energy and rate index for the 0.25 SA dose treatment (89.2%, 89.2% and 76.5%, respectively) were higher than those for the 1.0 SA dose treatment (80.8%, 78.3% and 54.3%, respectively). Salicylic acid doses, under 50 NaCl concentration exhibited higher germination percentage, energy and rate index values than under 100 NaCl. 0.25 SA dose, under 50 NaCl and 100 NaCl concentrations exhibited higher germination percentage, energy and rate index values than other doses. It was determined that increasing SA and salt doses negatively affected lentil germination.

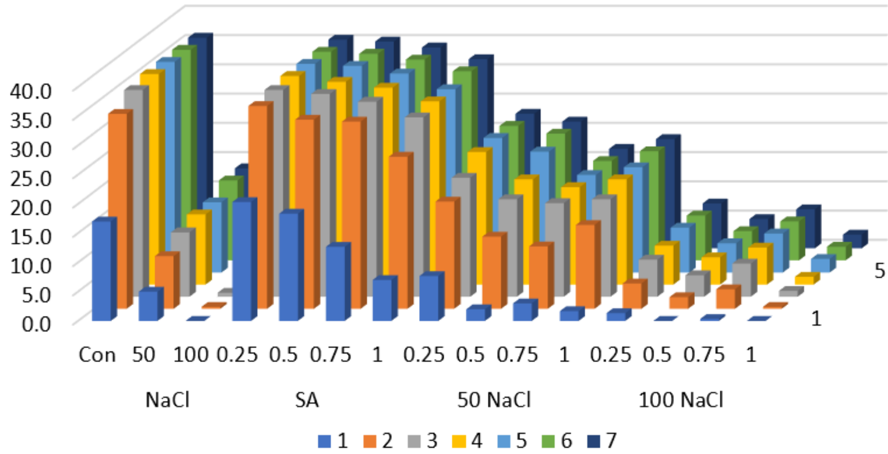


Figure 1. Effect of salt and SA treatments for germinated seeds and days.

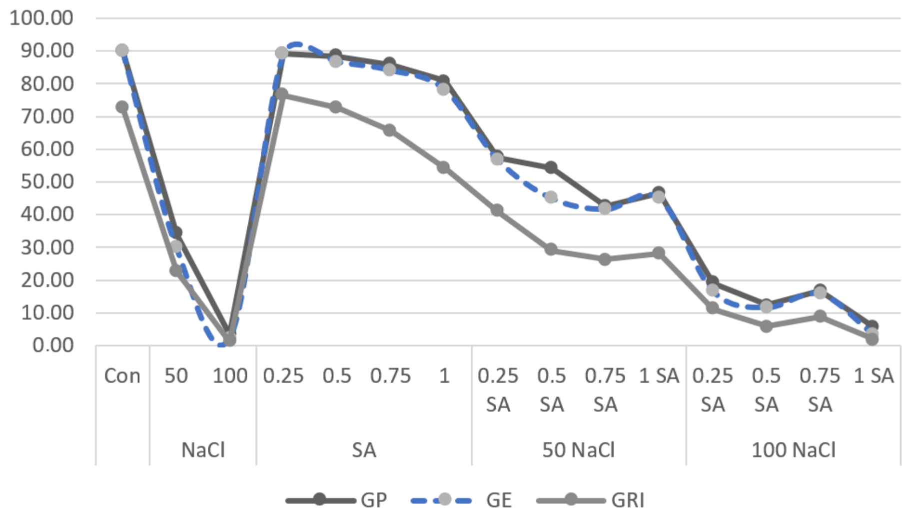


Figure 2. Effect of salt and SA treatments on germination percentage (GP), germination energy (GE), and germination rate index (GRI).

Table 2. Anova analysis for germination traits

Source	DF	GP	GE	GRI	MGT	PV
Treatment	14	3169.31**	3215.32**	2258.71**	0.293428**	14.3092**
Error	30	57.36	45.97	18.11	0.034870	0.3222
C. Total	44					

GP= Germination percentage %, GE= Germination energy %, GRI= germination rate index, MGT= Mean germination time, PV= Peak Value, VI= Vigour index %, *= P<0.05, **= P<0.001.

Ekmekci et al. (2005) reported that the decrease in germination rate with increasing salt dose was due to the toxicity of Na and Cl ions and the increase in osmotic pressure, which prevented the entry of water into the seed needed for germination. Uyanik et al. (2014) and Foolad and Lin (1997) reported that some metabolic disorders and inhibition of germination regulatory protein synthesis in plants exposed to salt stress caused a decrease in germination rate. It was also found by Mahdavi and Sanavy (2007), Fallahi et al. (2015), Gheidary et al. (2017), Haileselasie and Gselasie (2012), Tsegay and Gebreslassie (2014) and others that germination rate decreased with increasing salinity.

Mean Germination Time (MGT) and Peak Value (PV) were given in Table 2, Figure 3. MGT ranged from 4.0 to 5.5 days across all treatments. Mean germination time was more than 4.5 days for 100 NaCl concentration and all SA doses under 100 NaCl concentration. The highest mean germination time (5.26 days) was observed in 1.0 SA doses under 100 NaCl concentration. The control group and salicylic acid doses had the highest peak values. Specifically, 0.25 SA (5.94) and 1.0 SA (6.27) had the highest peak values. Altuner et al. (2022) found that salt applications decreased the germination index in all varieties and decreased to the lowest level in 150 mM salt applications. Additionally, the average germination time increased with increasing salt doses. Similarly, Petrović et al. (2016) and Anaya et al. (2018) reported negative effects of increasing salt stress on germination in legumes. It is worth noting that SA can act as a signaling molecule, activating defense mechanisms in seeds that help them tolerate salt stress. High concentrations of SA can inhibit germination or have no significant effect, although it can indirectly lead to improved water uptake and faster germination in salt-stressed environments. The effectiveness of SA can vary depending on the type of seed, the level of salt stress, and other environmental factors.

Radicle length, hypocotyl length and vigor index were given in Table 3 and Figure 4.

Table 3. Analysis of variance for radicle and hypocotyl length, radicle and hypocotyl fresh weight and vigor index

Variation Sources	DF	Radicle fresh weight	Hypocotyl fresh weight	DF	Radicle length	Hypocotyl length	Vigor Index
Treatment	4	0.000090	0.000073	9	11.5764**	5.29765**	2655.53**
Error	10	0.000061	0.000025	18	0.4355	0.06657	45.31
C. Total	14			27			
Treatment		Radicle fresh weight (g)	Hypocotyl fresh weight (g)				
Control		0.058	0.041				
0.25 SA		0.058	0.031				
0.50 SA		0.054	0.028				
0.75 SA		0.066	0.031				
1SA		0.066	0.035				

SA= Salicylic acid, *= P<0.05, **= P<0.001.

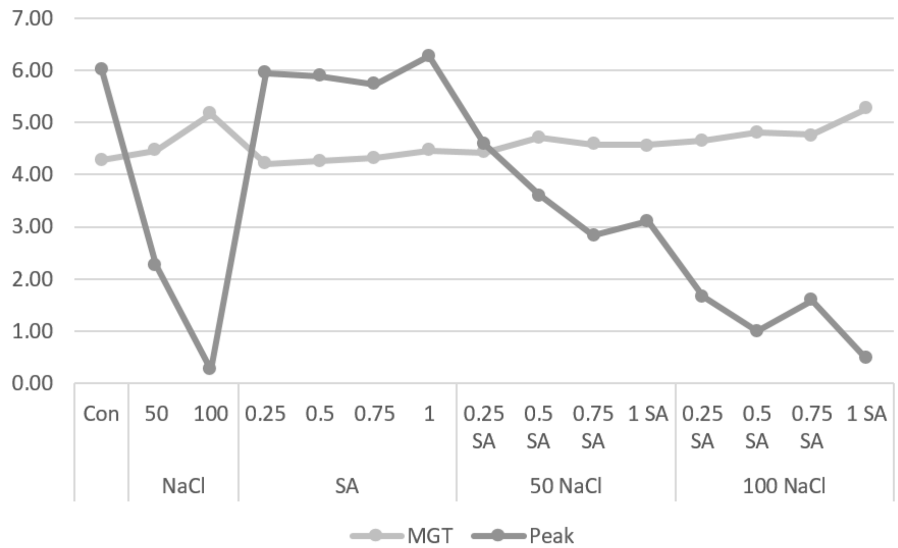
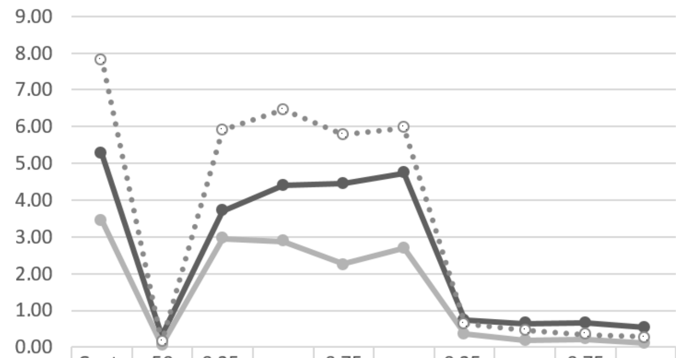


Figure 3. Effect of salt and SA treatments on radicle length, hypocotyl length, and vigor index on 8th days.



	Control	50 NaCl	0.25 SA	0,5 SA	0.75 SA	1 SA	0.25 SA	0.5 SA	0.75 SA	1 SA
			SA				50 NaCl			
Radicle length (cm)	5.25	0.31	3.70	4.40	4.45	4.73	0.74	0.65	0.65	0.55
Hypocotyl length (cm)	3.45	0.05	2.95	2.88	2.27	2.70	0.35	0.18	0.22	0.12
Vigor Index	7.83	0.13	5.92	6.45	5.79	5.97	0.63	0.45	0.33	0.28

Figure 4. Effect of salt and SA treatments on radicle length, hypocotyl length, and vigor index on 8th days.

Radicle length, hypocotyl length, and vigor index were measured for the control, 50 NaCl concentration, SA doses, and salicylic acid doses under 50 NaCl concentration. The results showed that high salt stress had a negative impact on radicle and hypocotyl lengths, even with different SA doses. The control group had the highest radicle length, hypocotyl length, and vigor index (5.25 cm, 3.45 cm, and 7.8, respectively). Salicylic acid doses appeared to increase radicle and hypocotyl length under 50 NaCl salt stress. Radicle and hypocotyl fresh weight were only measured at different doses of salicylic acid. According to Haileselasie and Gselasie (2012), radicle and hypocotyl lengths are crucial parameters for assessing salt stress. This is because radicles absorb salt from the soil and water and contribute to hypocotyl development. Therefore, measuring radicle and hypocotyl lengths provides initial information on the level of salt exposure experienced by plants. El-Tayeb (2005) and Khodary (2004) found that pre-treating plants with SA increased their weight. Fallahi et al. (2015) reported that an increase in salt content led to a decrease in the fresh weights of rootlets and stems.

5. Conclusion

The study indicated that different combinations of salicylic acid (SA) and salt (NaCl) concentrations on lentil germination had a crucial impact. The results showed that the germination percentage, energy, and rate index values were differently affected by the combination of SA and salt concentrations. The group exposed to the combination of salt and SA had different germination values compared to the control group. However, the germination values were not as low as those observed in the group exposed to 100 NaCl concentration alone. High salt doseshinder nutrient uptake and water absorption, further compromising the overall growth and vigor of the seeds.

It was found that the germination percentage, energy, and rate index values were higher for the 0.25 SA dose treatment compared to the 1.0 SA dose treatment. This suggests that a low dose of SA had a more positive effect on lentil germination under 50 NaCl concentration. The study also revealed that the effects of SA doses varied depending on the salt concentration. Under 50 NaCl concentration, the germination percentage, energy, and rate index values were higher for all SA doses compared to under 100 NaCl concentration. This indicates that under the lower salt concentration, SA has a more beneficial impact on lentil germination. Overall, increasing doses of SA and salt had a negative influence on lentil germination. However, the combination of SA and salt concentrations, as well as the specific doses used, played a significant role in determining the extent of this negative impact. Higher SA doses lead to reduced radicle growth and shoot development in lentil seedlings.

In addition to the negative effect on the germination of lentil seeds, it was worth noting that the increase in SA (salicylic acid) and salt doses can also impact other aspects of seed development.

These findings showed that significance the treatments of SA under salt stress conditions in lentil cultivation to ensure optimal seed germination and subsequent plant growth.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	G.K.T.	B.T.B.	F.B.
C	35	35	30
D	35	35	30
S		100	
DCP	50		50
DAI	50	25	25
L	50	30	20
W	50	30	20
CR	35	35	30
SR	50	30	20
PM	35	35	30
FA	45	10	45

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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