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EFFECTS OF DIFFERENT SALT STRESS AND TEMPERATURE APPLICATIONS ON GERMINATION IN MUNG BEAN (*Vigna Radiata* (L.) R. WILCZEK) GENOTYPES

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Abstract: Abiotic stress factors are among the primary elements hindering plant growth and development. Initial growth and development in plants are significantly affected by temperature and salinity. The study aimed to investigate the growth and development parameters of two different mung bean genotypes under different salt concentrations and temperatures. Four different salt doses (0, 5, 10, and 15 EC) and three different temperatures (15, 20, and 30°C) were used in the study. Germination percentage, shoot and root lengths, shoot fresh and dry weights, and ion leakage parameters were examined in the study. As a result of the study, the highest germination rate, shoot and root lengths, and fresh and dry weights were determined at 30°C under control, 5 EC salt applications, while the lowest was recorded at 15°C under 15 EC salt applications. The lowest ion leakage was determined in the control application at 15°C, while the highest was observed at 30°C under 15 EC applications. Increasing temperature positively affected growth parameters. It was determined that salt stress could be tolerated up to a certain level with high temperatures. This study conducted on mung bean is indicative of developing varieties tolerant to temperature and salt stress, which are important issues today.

Keywords: Abiotic stress, Temperature, Mung bean, Germination

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1. Introduction

Seed germination is a crucial agricultural characteristic with profound effects on plant growth and productivity (Demirkaya et al., 2017). Biotic factors such as seed structure and environmental factors significantly influence the germination and growth process. Additionally, there exists a range of abiotic factors that substantially affect seed germination and development, including drought, nutrient deficiencies or excesses, salinity, extreme temperatures, terrestrial and atmospheric pollution, and radiation (Dadaşoğlu and Ekinci, 2013; Çakmakçı and Dallar, 2019; Demirkaya et al., 2020). Abiotic stress factors significantly limit agricultural production on a global scale (Kopecká et al., 2023). Among abiotic stressors, salt stress negatively affects plant growth and development (Okumuş et al., 2023). High salt concentrations hinder water uptake during the initial stages of seed development, slow down embryo growth and development, and prolong the germination process (Munns and Gilliham, 2015). High salt concentrations reduce the water potential inside seeds, leading to osmotic stress, which hinders seed embryo hydration and complicates germination (Shabala and Pottosin, 2014).

Salinity directly affects plants through osmotic and ion stress, while its indirect effects result from structural damage in plants and the synthesis of toxic compounds due to these stress factors. Effects of salt stress include the synthesis of AOT (accumulation of organic osmolytes and toxic ions) compounds, metabolic toxicity, inhibition of photosynthesis, prevention of K+ uptake, and cell death, which disrupt DNA, protein, chlorophyll, and membrane function (Botella et al., 2005; Hong et al., 2009; Çakmakçı and Dallar, 2019). The germination characteristics of seeds can vary significantly depending on the plant species and environmental conditions, especially under different temperature regimes. Different temperature levels can have notable effects on germination rate, percentage, and subsequent seedling development (Kaya et al., 2006). Temperature stress can substantially reduce seed germination percentage, germination time, and seedling vigor in many plants (Lamichaney et al., 2021). High temperatures lead to cellular dehydration, causing a reduction in cell size and ultimately a decrease in growth (Arun-Chinnappa et al., 2017).

In this study, the effect of different salt stress and temperature applications on germination in mung bean

(*Vigna radiata* (L.) R. Wilczek) was investigated. Mung bean is an annual plant belonging to the legume family commonly used in world agriculture (Ram et al., 2019). Mung bean is rich in protein (22-28%), fiber, and minerals, and it is also abundant in magnesium, iron, B vitamins, and folate (Singh et al., 2017). Besides its high nutritional content, mung bean is a highly digestible legume (Benlioğlu and Özkan, 2020). Mung bean typically grows erect and can reach heights ranging from 30 to 90 cm, with flowers of various colors such as white and yellow (Huyen et al., 2014). Cultivation of mung bean in temperate regions is characterized by its short vegetative period and broad adaptability (Hanumantha Rao et al., 2016). Due to its low production cost and rich nutrient content, mung bean can be cultivated as an alternative crop in areas where protein sources are limited (Benlioğlu and Özkan, 2020). It is crucial to cultivate high-nutrient plant species under the influence of abiotic stress factors. Salinity and temperature are significant factors affecting early seedling development. The study aimed to investigate the effects of salinity and temperature on seed germination in mung bean.

2. Material and Methods

In this study, the tolerance to temperature and salinity was examined. The experiment was three-factorial and set up according to a split-split plot design in randomized complete blocks. As plant material, two different mung bean genotypes obtained from Argentina (Genotype 1) and Antalya (Genotype-2) were used. The study involved 3 different temperatures, 2 mung bean genotypes, and 3 different salt concentrations, conducted with 3 replications NaCl (Merck, Germany) was used for salt stress in the study. The salt levels were adjusted to control (0 EC), 5 EC, 10 EC, and 15 EC. The study was conducted at controlled conditions of 15°C, 20°C, and 30 °C in incubator.

The seeds to be used in the study were sterilized with 1% sodium hypochlorite for 5 minutes followed by rinsing with distilled water three times. The seeds were sown between 3 filter papers in groups of 25 and sealed with a ziplock bag to prevent moisture loss. 7 mL of solution was added to each filter paper. Seeds were considered germinated when the root (≥2 mm) emerged, and germinated seeds were counted for 14 days. Germination percentage (number of germinated seeds/25 x 100) was calculated at the end of the 14th day, and shoots and root length, fresh weight, dry weight, and ion leakage data were examined in randomly selected 10 seedlings.

Ion leakage was measured according to the method described by Aydın (2018). After washing with distilled water, the fresh shoots (0.5 g) were kept in 10 ml of distilled water at room temperature for 24 hours to measure the EC of the solution (O.D1). Then, after being autoclaved at 121°C for 20 minutes and cooled, the EC was measured again (O.D2), and ion leakage in leaf tissues was calculated using the following equation (Aydın, 2018).

% Ion leakage = (O.D1 / O.D2) x 100

2.1. Statistical Analysis

The statistical analysis was conducted using the factorial experimental design with four replications in a randomized complete block design. The data obtained from the study were analyzed using the "JMP 13.2.0" software according to the factorial experimental design in randomized complete blocks. The treatment means were compared using Tukey's Honestly Significant Difference (HSD) test (Snedecor and Cochran, 1967). Also, PCA and correlation analyses were carried out JMP 13.2.0 software.

3. Results and Discussion

Upon examination of the features considered in the experiment, statistically significant differences between the applications were found. Additionally, the temperature*salt interaction was found to be statistically significant at the 5% level. In the genotype originating from Argentina, Genotype-1, the highest germination rate was determined to be 100% at 30°C under control conditions and with a 5 EC salt application, while the lowest rate was 23% at 15°C with a 15 EC salt application. However, a significant increase in germination rate of cowpea seeds was observed under high-temperature conditions. Specifically, the germination rate determined at 30°C with a 15 EC salt application (76%) was higher than that obtained under control conditions at 15°C (69%). It was determined that under high temperatures, salt stress could be tolerated up to a certain level.

Similar results were also recorded for shoot length and root length. The lowest values for shoot length and root length were determined in the applications at 15°C. The highest shoot length (13.08 cm) was determined at 30°C in the control group, followed by a 10.53 cm shoot length in the 5 EC salt applications at 30°C, with the control group at 20°C having a lower shoot length (9.06 cm). Root length, on the other hand, was found to be highest at 30°C and 20°C with values exceeding 10 cm, followed by the 5 EC salt applications at 30°C and 20°C. It is evident that shoot and root formation performance is significantly lower under low-temperature conditions.

When examining fresh and dry weights, the highest fresh weight was determined as 5492 mg in the control application at 30°C and 5250 mg in the control application at 20°C, while the lowest was determined as 19.50 mg in the 15 EC salt applications at 15°C. In terms of dry weight, it was determined as 430.53 mg in the control application at 30°C and 413.58 mg in the control application at 20°C, while the lowest was determined as 2.05 mg in the 15 EC salt applications at 15°C. Ion leakage was evaluated, with the highest percentage of 58.93% observed in the 15 EC application at 30°C, and the lowest percentage of 34.25% observed in the control application at 15°C (Table 1).

Table 1. Germination Rate (%), Shoot and Root Length (cm), Fresh and Dry Weights (mg), and Ion Leakage (%) of Genotype-1 at Different Temperature and Salt Concentrations

Temperature	Treatment	Germination Rate (%)	Shoot Length (cm)	Root Length (cm)	Fresh Weight (mg)	Dry Weight (mg)	Ion Leakage (%)
15ºC	Control	69.00±1.17f	0.82±0.11h	0.77±0.19de	193.75±56.4fg	25.10±11.2ef	34.25±0.87i
	5 EC	51.00±1.17h	0.56±0.11hi	0.62±0.19de	127.25±56.4fg	20.60±11.2ef	41.03±0.87fgh
	10 EC	45.00±1.17i	0.29±0.11i	0.38±0.19de	51.75±56.4g	6.05±11.2f	46.62±0.87de
	15 EC	23.00±1.17j	0.16±0.11i	0.15±0.19e	19.50±56.4g	2.05±11.2f	48.43±0.87cd
Mean		47.00	0.46	0.48	98.06	13.45	45.58
20ºC	Control	88.00±1.17b	9.06±0.11c	10.43±0.19a	5250.00±56.4ab	413.58±11.2a	38.66±0.87gh
	5 EC	82.00±1.17cd	7.25±0.11d	7.70±0.19b	4680.00±56.4c	384.70±11.2a	42.74±0.87efg
	10 EC	79.00±1.17de	3.23±0.11f	4.50±0.19c	3735.00±56.4d	326.13±11.2b	50.91±0.87bcd
	15 EC	58.00±1.17g	1.88±0.11g	1.05±0.19de	513.25±56.4fg	73.00±11.2de	55.12±0.87ab
Mean		76.75	5.36	5.92	3544.56	299.35	46.86
30ºC	Control	100.00±1.17a	13.08±0.11a	10.75±0.19a	5492.50±56.4a	430.53±11.2a	37.54±0.87hi
	5 EC	100.00±1.17a	10.53±0.11b	7.90±0.19b	4800.00±56.4bc	409.50±11.2a	43.99±0.87ef
	10 EC	87.00±1.17bc	4.49±0.11e	4.31±0.19c	2345.00±56.4e	166.75±11.2c	52.47±0.87bc
	15 EC	76.00±1.17e	2.28±0.11g	1.24±0.19d	610.00±56.4f	83.63±11.2d	58.93±0.87a
Mean		90.75	7.60	6.05	3311.88	272.60	48.23
Temperature x Salt *P<0.05; **P<0.01		**	**	**	**	**	*

In the 2nd genotype originating from Antalya, the highest germination rate was determined to be 87% under the control condition at 30°C, while the lowest was 25% under the application of 15 EC salt at 15°C. Significant increases in germination rate at high temperatures were observed, like those originating from Argentina. When shoot and root lengths were examined, the highest shoot length was determined to be 12.07 cm under the control condition at 30°C and 8.93 cm under 5 EC application, whereas the lowest was 0.14 cm under the application of 15 EC salt at 15°C. Regarding root length, the highest was determined to be 10.30 cm under the control condition at 30°C and 9.79 cm at 20°C under the control condition. When considering fresh and dry weights, the highest fresh weight was 5010 mg under the control condition at 30°C and 4474 mg at 20°C, while the lowest was 18 mg under the application of 15 EC salt at 15°C. In terms of dry weight, it was 479.25 mg under the control condition at 30°C and 420 mg at 20°C under the control condition, with the lowest being 1.83 mg under the application of 15 EC salt at 15°C. Ion leakage was evaluated, with the highest being 53.84% under the application of 15 EC at 30°C, and the lowest being 30.53% under the control condition at 15°C (Table 2).

Table 2. Germination rate (%), shoot and root length (cm), fresh and dry weights (mg), and ion leakage (%) of genotype-2 at different temperature and salt concentrations

Temperature	Treatment	Germination Rate (%)	Shoot Length (cm)	Root Length (cm)	Fresh Weight (mg)	Dry Weight (mg)	Ion Leakage (%)
15ºC	Control	65.00±1.28ef	0.64±0.15f	0.70±0.20d	173.00±47.77e	17.50±3.16fg	30.53±0.96h
	5 EC	57.00±1.28g	0.52±0.15f	0.54±0.20d	105.25±47.77e	11.57±3.16g	37.64±0.96fg
	10 EC	39.00±1.28i	0.26±0.15f	0.30±0.20d	48.00±47.77e	5.00±3.16g	41.62±0.96ef
	15 EC	25.00±1.28j	0.14±0.15f	0.13±0.20d	18.00±47.77e	1.83±3.16g	44.57±0.96de
Mean		46.5	0.39	0.42	86.06	8.98	38.60
20ºC	Control	79.00±1.28bc	8.66±0.15b	9.79±0.20a	4474.25±47.77b	420.00±3.16b	34.12±0.96gh
	5 EC	73.00±1.28cd	6.85±0.15c	7.26±0.20b	3879.50±47.77c	372.50±3.16c	42.73±0.96de
	10 EC	59.00±1.28fg	2.77±0.15d	4.13±0.20c	3187.50±47.77d	307.50±3.16d	47.36±0.96cd
	15 EC	50.00±1.28h	1.65±0.15e	1.01±0.20	408.75±47.77e	42.87±3.16f	52.25±0.96ab
Mean		65.25	4.98	5.54	2987.50	285.72	44.11
30ºC	Control	87.00±1.28a	12.07±0.15a	10.30±0.20a	5010.00±47.77a	479.25±3.16a	37.47±0.96fg
	5 EC	81.00±1.28ab	8.93±0.15b	7.40±0.20b	4617.50±47.77ab	430.50±3.16b	41.46±0.96ef
	10 EC	68.00±1.28de	3.52±0.15d	3.85±0.20c	2797.50±47.77d	215.25±3.16e	48.05±0.96bc
	15 EC	58.00±1.28g	1.80±0.15e	1.14±0.20d	413.25±47.77e	47.48±3.16f	53.84±0.96a
Mean		73.50	6.58	5.67	3209.56	293.12	42.21
Temperature x Salt *P<0.05; **P<0.01		*	**	**	**	**	*

In the study, the effect of 4 different salt doses (0, 5, 10, and 15 EC) and 3 different temperatures (15, 20, and 30°C) on germination parameters was supported by PCA analyses. According to the calculated PCA analysis, the graphs explain the applications by 93% (Component 1 %77.9; Component 2 %15.3). Upon examination of the obtained findings, a linear relationship between temperature and mung bean germination is evident. With increasing temperature, there is an increased tendency for root and shoot formation (Figure 1). With increasing temperature, the application of low doses of salt is positioned at the same place as the control. Looking at the ion leakage graph, it can be observed that salt applications are positioned in the opposite direction to germination parameters. As the salt application in the environment increases, so does the ion leakage (Figure 1).

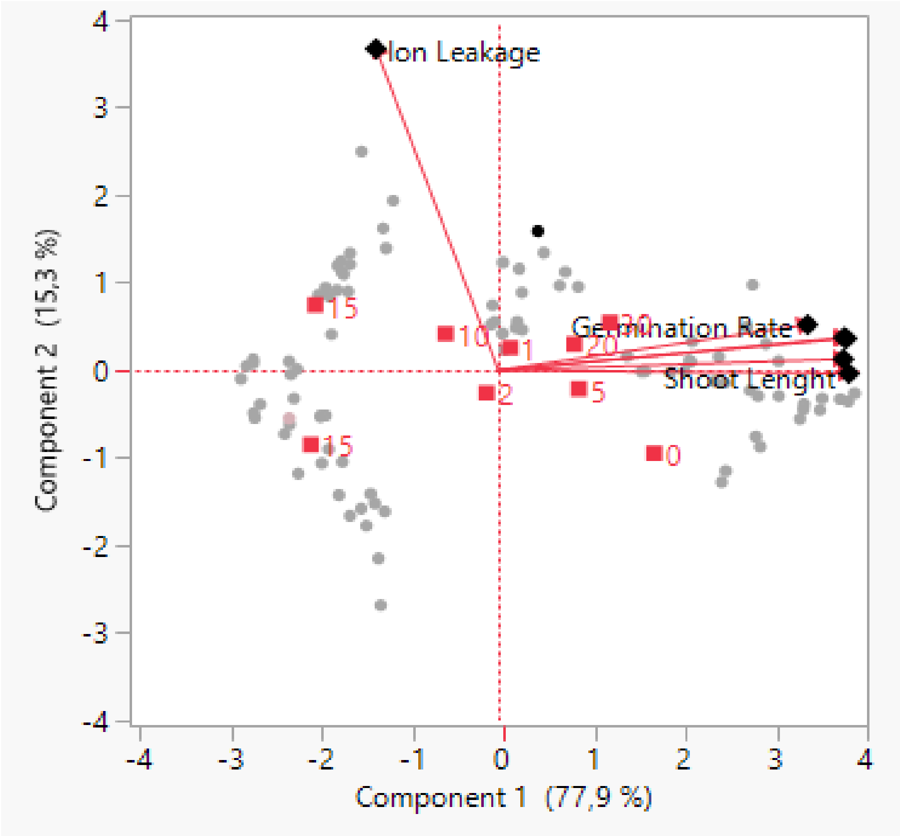


Figure 1. Principal Component Analysis (PCA) between germination parameters of mung bean with different salt and temperature treatments.

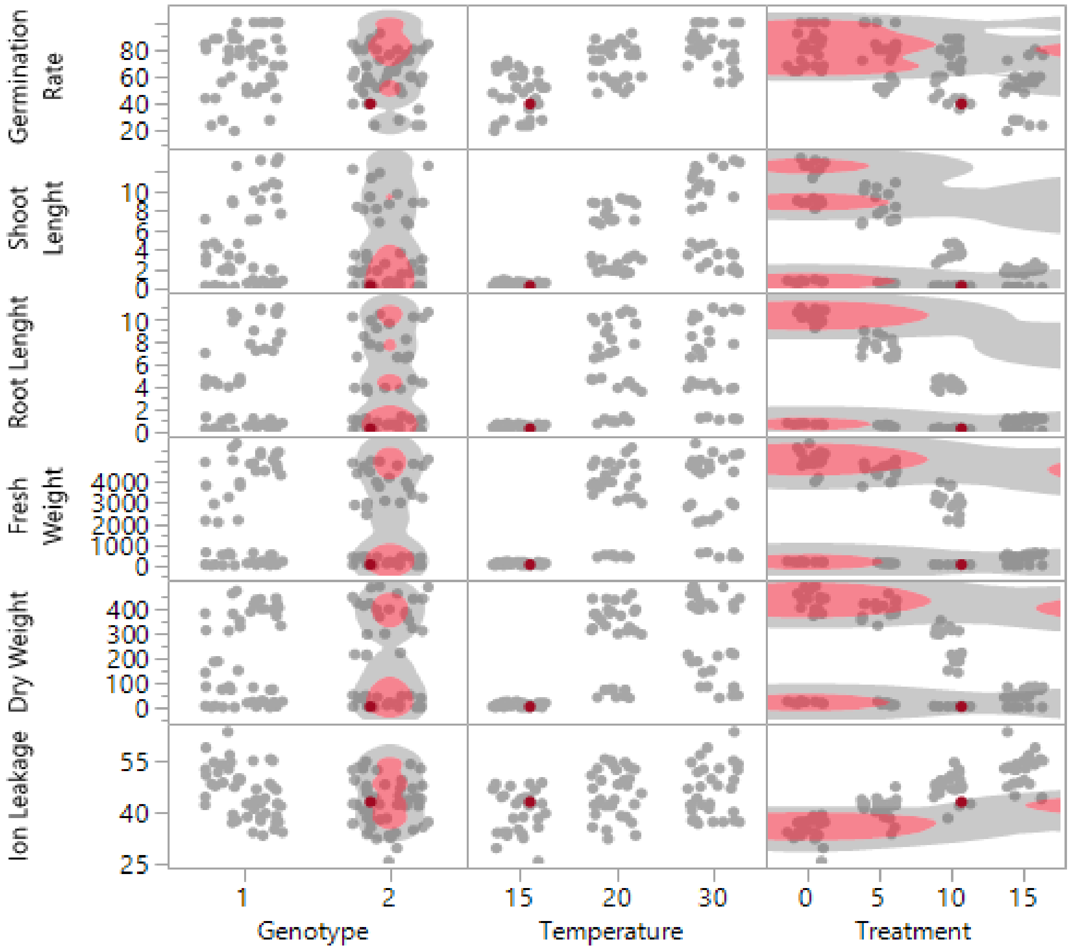
The PCA graph, which evaluates the interaction of variety, salt, and temperature treatments on the germination parameters of mung bean seeds, also supports the findings obtained (Figure 2). While germination rates vary according to variety, germination parameters are positively affected under conditions where low salt and high temperature treatments are applied together. Under high temperature conditions, the application of low salt doses has yielded results similar to the control.

Salt and temperature stress significantly reduces the growth and development of plants during their initial growth stages. Salt and temperature stress can directly lead to plant death and simultaneously significantly reduce plant growth, seed germination, root and shoot length (Yılmaz et al., 2023). When both genotypes were examined, an increase in germination rates was observed with increasing temperatures. In parallel, plant growth and development were negatively affected by increasing salt doses. In other studies, in beans (*Phaseolus vulgaris* L.) (Kibar et al., 2020), they found that increasing salt doses (0, 50, 100, and 200 mM) resulted in a decrease in germination rate from 88.16% to 31.11% and significant decreases in other parameters such as shoot and root length, as well as fresh and dry weights. In maize (*Zea mays* L.) (Çakmakçı and Dallar, 2019), they observed a decrease in germination rate from 81.27% to 45.87% with increasing temperatures and concluded that increasing temperature and salt application negatively affected growth and development. In beans (*Phaseolus vulgaris* L.) (Yılmaz et al., 2023), the effect of 18 local genotypes and a 200 mM salt concentration on growth and development was investigated, and it was found that the salty environment reduced germination by 38%. Salt stress negatively affects shoot and root lengths (Saha et al., 2010). Root length ensures more efficient utilization of soil minerals and water by the plant; therefore, root length is an important parameter during initial growth and development (Rabie, 2005). Significant decreases in shoot and root lengths were observed at high salt doses in both genotypes. Similar decreases have been observed in other studies (Çakmakçı and Dallar, 2019). Most mung bean genotypes exhibit tolerance to moderate salt levels (9-18 mhos cm) (Singh and Singh, 2011). In the obtained results, resistance was observed up to 15 EC salt at 20 and 30°C. This finding is consistent with similar studies (Benlioğlu and Özkan, 2020).

Both fresh and dry weights were similarly negatively affected by low temperature and high salt density. In other studies, it has been noted that increasing salt densities not only affect shoot and root lengths but also lead to decreases in fresh and dry weights (Misra and Dwivedi, 2004).

Salinity causes yield losses in more than 50% of the world's arid and semi-arid regions (Naeem et al., 2020). The high osmotic effect, ion toxicity, oxidative stress, and nutrient deficiencies in these areas adversely affect plant growth (Naeem et al., 2020). High salt concentrations lead to membrane breakdown, increasing ion leakage (Kalisz et al., 2023). The highest ion leakage values were observed in G1 and G2 under 15 EC application at 30°C, while the lowest was observed in G1 and G2 under 10°C control application. A lower ion leakage value indicates higher tolerance to salt stress (Okumuş et al., 2023). In a study on red clover, ion leakage ranged from 31.74% to 68.36% with increasing salt doses (Okumuş, 2022). Kurt et al., in their study on soybean (*Glycine max*) in 2023, reported that ion leakage in leaf tissues increased with increasing salt doses.

In other studies, it has been found that ion leakage increases with increasing salt concentrations, and the reason for this is reported to be the parallel increase in ion leakage with salt doses, resulting from damage to cell membrane integrity and stability (Oral et al., 2020).



	Germination rate	Shoot lenght	Root lenght	Fresh weight	Dry weight	İon leakage
Germination rate	1,0000	0,8195	0,7966	0,8034	0,7861	-0,1928
Shoot lenght	0,8195	1,0000	0,9620	0,9286	0,9220	-0,2965
Root lenght	0,7966	0,9620	1,0000	0,9678	0,9585	-0,3403
Fresh weight	0,8034	0,9286	0,9678	1,0000	0,9883	-0,2466
Dry weight	0,7861	0,9220	0,9585	0,9883	1,0000	-0,2416
İon leakage	-0,1928	-0,2965	-0,3403	-0,2466	-0,2416	1,0000

Figure 2. Scatterplot matrix and correlation of the germination parameters of mung bean with different salt and temperature treatments.

4. Conclusion

Salinity and high temperature are significant abiotic stress factors that adversely affect plant growth and productivity, limiting agricultural production. Bean species are among the most sensitive vegetables to salinity. In this study, the effects of different salt and temperature treatments on germination parameters in mung beans were investigated. The research revealed that salinity greatly inhibited germination in mung bean seeds. As salt concentration increased, a significant decrease in germination rate was observed. With increasing salt stress, there was an elevation in ion leakage, accompanied by ion toxicity. It is believed that this results in the blockage of water passage in seeds and limits seed germination as the salt concentration in the environment increases.

Similar results were obtained with the two genotypes used in the study. Additionally, it was observed that the damage caused by salt stress decreased with increasing temperature. This study, designed with two different genotypes, aimed to determine the germination characteristics of mung beans. However, it is a fundamental study and provides guidance for drought and salinity-resistant variety breeding, in line with sustainable development goals, responsible production and consumption, climate action, and addressing global climate issues.

Author Contributions

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

	O.O.	A.D.S.
C	50	50
D	50	50
L	50	50
W	50	50
CR	50	50
SR	50	50

C=Concept, D= design, L= literature search, W= writing, CR= critical review, SR= submission and revision.

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. The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to.

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