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THE SINGLE AND INTERACTIVE EFFECT OF SALINITY AND TEMPERATURE ON GERMINATION CHARACTERISTICS OF ITALIAN RYEGRASS (*Lolium multiflorum* LAM.) SEEDS

Onur OKUMUŞ[1], Akife DALDA ŞEKERCİ[2]*, Satı UZUN[1]

[1]*Erciyes University, Faculty of Agriculture, Department of Field Crops, 38039, Kayseri, Türkiye*
[2]*Erciyes University, Faculty of Agriculture, Department of Horticulture, 38039, Kayseri, Türkiye*

Abstract: Italian ryegrass (*Lolium multiflorum*) is a grass species within the *Lolium* genus of the Poaceae family. In recent years, annual ryegrass has shown excellent adaptability to the climatic and soil conditions of Türkiye. It serves as a good alternative forage source to bridge the forage deficit and is widely used as a low-growing native turf mixture in local landscaping. Abiotic stress factors are among the primary elements that hinder plant growth and development. Temperature and salinity significantly affect seed germination and development. This study aimed to investigate the germination and growth parameters of three different Italian ryegrass varieties (İlkadım, Kocayaşar, Zeybek) under different salt concentrations (Sodium chloride-NaCl) and temperatures. Three different salt doses (control, 5 EC, and 10 EC) and three different temperatures (15 °C, 20 °C, and 30 °C) were used in the study. Germination percentage, shoot and root lengths, fresh and dry weights of shoots, and ion leakage parameters were examined. The results showed that the highest germination rate, shoot and root lengths, and fresh and dry weights in all varieties were recorded at 20 °C with 0 EC and 20 °C with 5 EC salt treatments, while the lowest were observed at 15 °C with 10 EC salt treatments. The lowest ion leakage was determined in the control treatment at 15 °C, while the highest ion leakage was observed in the 10 EC treatment at 30 °C. Increasing temperature positively influenced growth parameters. It was determined that salt stress could be tolerated up to a certain level at higher temperatures. This study on different Italian ryegrass varieties highlights the importance of developing ryegrass varieties resistant to temperature and salt stress, which are significant issues in sustainable agriculture.

Keywords: Abiotic stress, Ion leakage, Italian ryegrass, Principal component analysis

***Corresponding author:** Erciyes University, Faculty of Agriculture, Department of Horticulture, 38039, Kayseri, Türkiye
E mail: akidal_@hotmail.com (A. DALDA ŞEKERCİ)
Onur OKUMUŞ https://orcid.org/0000-0001-6957-3729
Akife DALDA ŞEKERCİ https://orcid.org/0000-0001-8554-6501
Satı UZUN https://orcid.org/0000-0001-9919-3145

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1. Introduction

Abiotic stress is a significant issue that greatly restricts agricultural production globally (Kopecka et al., 2023). One of the abiotic stress factors, salinity, is considered one of the most critical environmental stresses that significantly reduce yield and quality in crop production worldwide (Doruk Kahraman and Topal, 2023). More than 800 million hectares of land globally are affected by salinity, constituting over 6% of the world's total land area, and this proportion is increasing due to natural and anthropogenic activities (Munns and Tester, 2008; Hasanuzzaman and Fujita, 2022). Salt stress causes physiological, biochemical, and metabolic changes in plants, leading to a decrease in crop production. The main components of salt stress in plants are osmotic stress, ionic stress, and secondary stress, namely the excessive accumulation of reactive oxygen species (ROS) (Morton et al., 2019; Li et al., 2022).

The germination characteristics of seeds at different temperatures can vary significantly depending on the plant species and environmental conditions (Okumuş et al., 2023). Different temperature levels can have noticeable effects on germination rate, percentage, and post-germination seedling development (Kaya et al., 2006; Okumuş et al., 2024). Temperature stress significantly reduces the seed germination percentage, germination time, and seedling vigor in many plants (Lamichaney et al., 2021). High temperatures lead to cell dehydration, causing cell size reduction and ultimately leading to decreased growth (Arun-Chinnappa et al., 2017).

Italian ryegrass (*Lolium multiflorum* Lam.) is a plant species within the genus *Lolium* of the Poaceae family, belonging to the order *Poales* (Lale and Kökten, 2020). In recent years, annual ryegrass has shown excellent adaptability to the climate and soil conditions in Türkiye and is expected to be a good alternative forage source to address the forage deficit (Özkan et al., 2022). According to the Turkish Statistical Institute (TÜİK) statistics, in 2022, the sown area was 539,944 decares, and green fodder production was 2,122,105 tons (TÜİK, 2023). The climates it best adapts to are cool and humid. It can easily be grown in areas with an annual rainfall of over 400 mm (Açıkgöz, 2021). The optimum air temperature for the most productive growth is between 18-24 °C (Pişkin, 2007).

Grass plays a significant role in human life by adding elegance to the environment and forming the basis of many recreational sports. They constitute a large part of both residential and commercial landscapes. One native landscaping option is the use of native grass mixtures, which provide a turf appearance with low-growing native grasses (Pooya et al., 2013). Native plants are resilient as they are adapted to local conditions (Butler et al., 2012). Because native plants require less maintenance, they offer excellent options for large commercial landscapes and residential gardens. Turfgrass breeders are in search of varieties that can grow satisfactorily across a wide range of climates, soil, and environmental conditions (Pessarakli and Kopec, 2008). These grasses are excellent candidates for producing dwarf and turf-type varieties and are also used as turfgrass in arid and semi-arid regions worldwide to develop more attractive and low-maintenance varieties. Due to these reasons, there is increasing interest in selecting and producing native grass varieties that exhibit many other beneficial characteristics (Bormann et al., 2001). Because of their different growth patterns, a mixture of two or more grass varieties can complement each other to provide both functional and aesthetic improvements in turf-grassquality.

Therefore, it is crucial to conduct sustainable agriculture-focused studies that adapt to global climate change. Plants' responses to salt and temperature stress vary among species and even among varieties, and adaptation to stress conditions needs to be evaluated on a species-specific basis. This study aims to determine the responses of some annual grass varieties to salt stress at different temperatures and to examine germination and some seedling development characteristics during the germination period under increasing NaCl concentrations.

2. Materials and Methods

This study investigated the temperature and salinity tolerances of three different Italian ryegrass varieties. The experiment was designed with three factors and established according to the split-split plot design in a randomized complete block design. The varieties used as plant material in the study were İlkadım, Kocayaşar, and Zeybek. İlkadım and Kocayaşar varieties were registered by the Black Sea Agricultural Research Institute, and Zeybek variety was registered by the Aegean Agricultural Research Institute.

In the study, NaCl (Merck, Germany) was used to induce salt stress. The salt levels were set to control, 5 EC, and 10 EC. The study was conducted under controlled conditions at temperatures of 15 °C, 20 °C, and 30 °C. The seeds used in the study were sterilized with 10% sodium hypochlorite for 5 minutes and then rinsed three times with distilled water. The seeds were placed in groups of 25 between three filter papers, and the edges were sealed with zip lock bags to prevent moisture loss. Seven milliliters of solution were added to each filter paper. Seeds were considered germinated when the root length reached ≥2 mm, and germinated seeds were counted for 14 days. At the end of the 14-day period, the germination percentage (Number of germinated seeds/25 x 100) was calculated. Additionally, shoot and root length, fresh weight, dry weight, and ion leakage data were examined on 10 randomly selected seedlings.

Ion leakage was measured according to the method described by Aydın (2018). Fresh shoots (0.5 g) were washed with distilled water and incubated in 10 ml of distilled water at room temperature for 24 hours, after which the solution's EC was measured (O.D1). The samples were then autoclaved at 121 °C for 20 minutes, cooled, and the EC was measured again (O.D2). Ion leakage in leaf tissues was calculated using the following formula Equation 1;

$$\% \text{ Ion leakage} = (\text{O.D1} / \text{O.D2}) \times 100 \quad (1)$$

2.1. Statistical Analysis

The research was established in a factorial design with four replications in randomized plots. The data obtained from the research were analyzed using the "JMP 13.2.0" software according to the factorial design in randomized plots. Treatment means were compared using the Tukey's Multiple Comparison Test (Snedecor and Cochran, 1967).

3. Results and Discussion

3.1. Germination Percentage

In this study, the germination percentages of three Italian ryegrass varieties at different salt doses and temperatures were determined. In terms of germination percentage, variety, salt dose, variety x salt dose, and variety x temperature were found to be statistically significant ($P<0.001$), while the interaction of variety x temperature x salt dose was found to be insignificant.

According to the results, the germination percentage ranged from 87.33% to 44.00% for the İlkadım variety, from 82.66% to 40.66% for the Kocayaşar variety, and from 92.00% to 51.33% for the Zeybek variety (Table 1). The differences in germination percentages among the varieties were statistically significant. The highest germination percentage for all three varieties was observed at 20 °C, while the lowest was at 15 °C. A decline in germination percentages was observed in the varieties with increasing salt doses. The results indicate that high temperature and salt levels delay the germination period. It is suggested that excessive salt ions, aided by temperature, may limit water uptake by the germinating seeds. As is well known, seed water uptake depends on the osmotic potential of the seed and its surrounding environment, and one of the reasons salinity adversely affects germination is osmotic stress (Doğan and Budaklı Çarpıcı, 2016).

Table 1. Germination rate (%), shoot and root length (cm), fresh and dry weights (mg), and ion leakage (%) of Italian ryegrass at different temperature and salt concentrations

°C	Salt Doses	Germination Rate			Ion Leakage		
		İlkadım	Kocayaşar	Zeybek	İlkadım	Kocayaşar	Zeybek
	Cont.	59.33±1.50hij	70.66±1.50	71.33±1.50fg	26.03±0.85n	29.63±0.85mn	32.33±0.85klm
15	5 EC	52.00±1.50ijk	60.00±1.50hi	64.00±1.50gh	34.27±0.85klm	39.47±0.85ij	41.80±0.85hi
	10 EC	44.00±1.50kl	40.66±1.50l	51.33±1.50jk	48.07±0.85c-f	48.37±0.85b-f	50.37±0.85a-e
	Cont.	87.33±1.50bc	82.66±1.50cd	99.33±1.50a	31.73±0.85lm	32.17±0.85lm	32.10±0.85lm
20	5 EC	83.33±1.50cd	70.66±1.50fg	92.00±1.50ab	42.70±0.85ghi	44.40±0.85fgh	44.40±0.85fgh
	10 EC	72.66±1.50ef	60.66±1.50h	81.33±1.50cd	51.90±0.85a-d	51.37±0.85a-e	51.36±0.85a-e
	Cont.	80.66±1.50cde	72.00±1.50fg	86.66±1.50bcd	34.17±0.85klm	35.50±0.85jkl	36.83±0.85jk
30	5 EC	71.33±1.50fg	56.00±1.50hij	78.66±1.50def	42.23±0.85ghi	47.07±0.85efg	47.93±0.85c-f
	10 EC	58.66±1.50hij	42.66±1.50l	70.66±1.50fg	52.80±0.85ab	52.37±0.85abc	53.46±0.85a
Mean Significant		TxC**	CxS **	TxCxS NS	TxC**	CxS *	TxCxS NS
°C	Salt Doses	Root Length			Fresh Weight		
		İlkadım	Kocayaşar	Zeybek	İlkadım	Kocayaşar	Zeybek
	Cont.	3.33±0.16ef	2.10±0.16hi	2.56±0.16 e-h	50.06±1.29ijk	50.43±1.29ijk	60.33±1.29fgh
15	5 EC	2.26±0.16gh	1.36±0.16ij	2.13±0.16hi	42.73±1.29l	43.63±1.29kl	57.13±1.29ghi
	10 EC	1.13±0.16j	0.90±0.16j	1.26±0.16ij	31.40±1.29n	29.27±1.29n	50.33±1.29ijk
	Cont.	3.13±0.16efg	5.50±0.16b	5.73±0.16b	73.10±1.29bc	66.43±1.29c-f	82.97±1.29a
20	5 EC	3.23±0.16ef	5.23±0.16b	4.23±0.16cd	64.60±1.29def	52.70±1.29ij	78.43±1.29ab
	10 EC	2.50±0.16fgh	3.40±0.16de	2.53±0.16e-h	51.33±1.29ij	44.17±1.29kl	61.30±1.29efg
	Cont.	4.86±0.16bc	5.57±0.16b	6.93±0.16a	64.60±1.29def	62.53±1.29efg	70.17±1.29cd
30	5 EC	2.86±0.16 e-h	3.37±0.16ef	5.30±0.16b	45.60±1.29jkl	53.93±1.29hi	68.30±1.29cde
	10 EC	2.50±0.16fgh	3.33±0.16ef	2.60±0.16 e-h	39.87±1.29lm	34.73±1.29mn	52.90±1.29i
Mean Significant		TxC**	CxS **	TxCxS **	TxC**	CxS **	TxCxS **

Table 1. Germination rate (%), shoot and root length (cm), fresh and dry weights (mg), and ion leakage (%) of Italian ryegrass at different temperature and salt concentrations (continue)

°C	Salt Doses	Shoot Length		
		İlkadım	Kocayaşar	Zeybek
	Cont.	4.03±0.18f-i	3.50±0.18hij	4.87±0.18ef
15	5 EC	3.60±0.18hij	3.26±0.18ij	4.37±0.18e-g
	10 EC	2.26±0.18kl	1.90±0.18l	2.83±0.18jkl
	Cont.	5.30±0.18de	6.37±0.18bc	6.77±0.18bc
20	5 EC	4.83±0.18ef	5.27±0.18de	5.13±0.18de
	10 EC	4.40±0.18e-h	3.50±0.18hij	3.63±0.18g-j
	Cont.	5.23±0.18de	7.20±0.18ab	8.00±0.18a
30	5 EC	4.60±0.18efg	5.97±0.18cd	4.80±0.18ef
	10 EC	2.98±0.18jk	2.83±0.18jkl	2.87±0.18jkl
Mean Significant		TxC**	CxS **	TxCxS **
°C	Salt Doses	Dry Weight		
		İlkadım	Kocayaşar	Zeybek
	Cont.	5.26±0.18h-k	5.43±0.18g-j	6.03±0.18fgh
15	5 EC	4.33±0.18kl	4.53±0.18jkl	5.43±0.18g-j
	10 EC	3.93±0.18l	2.50±0.18m	5.23±0.18 h-k
	Cont.	8.30±0.18ab	7.17±0.18cde	8.87±0.18a
20	5 EC	6.73±0.18def	5.60±0.18ghi	8.07±0.18abc
	10 EC	5.46±0.18g-j	5.10±0.18h-k	6.37±0.18efg
	Cont.	6.93±0.18def	6.73±0.18	7.73±0.18bcd
30	5 EC	4.97±0.18ijk	6.07±0.18fgh	7.30±0.18b-e
	10 EC	3.70±0.18l	3.70±0.18l	5.63±0.18ghi
Mean Significant		TxC**	CxS **	TxCxS **

Previous studies have also indicated a direct relationship between the inhibitory effect of salt ions on seed germination and the hindrance of embryo growth with increasing temperature.

In similar studies, Soysal et al. (2021) reported that salt stress predominantly had a reducing effect on germination in their experiment with an annual ryegrass variety. Kuşvuran et al. (2015) reported that 150 and 200 mM NaCl adversely affected germination in their study with *Lolium perenne*. Another study reported that tetraploid Italian ryegrass (GT) had higher germination percentage and germination energy under different salinity levels and temperatures compared to diploid Italian ryegrass, and the most suitable temperature for seed germination was determined to be 25°C (Özkan et al. 2022). In a related study, the optimal germination percentage and germination energy were determined to be at 25°C, indicating that grass species prefer relatively higher temperatures for seed germination (Lin et al., 2018).

3.2. Ion Leakage

In terms of ion leakage, variety and variety x temperature were found to be significant at the 0.01 level, while variety x salt dose was significant at the 0.5 level, and the interaction of variety x temperature x salt dose was found to be insignificant.

As seen in Table 1, ion leakage ranged from 52.80% to 26.03% for the İlkadım variety, from 52.37% to 29.63% for the Kocayaşar variety, and from 53.46% to 32.33% for the Zeybek variety. An increase in ion leakage was observed with increasing salt doses and temperatures. The high osmotic effect, ion toxicity, oxidative stress, and nutrient deficiencies in these areas adversely affect plant growth (Naeem et al., 2020; Okumuş and Dalda-Şekerci, 2024). High salt concentrations lead to membrane breakdown, increasing ion leakage (Kalisz et al., 2023).

3.3. Shoot and Root Lengths

The analysis revealed that the effects of variety, salt dose, variety x salt dose, variety x temperature, and variety x salt dose x temperature interactions on shoot and root lengths were statistically significant at the 1% level. According to Table 1, shoot lengths varied between 2.26 cm and 5.30 cm for the İlkadım variety, 1.90 cm and 7.20 cm for the Kocayaşar variety, and 2.83 cm and 8.00 cm for the Zeybek variety, with the highest shoot length observed at 30°C. For root length, the İlkadım variety ranged from 1.13 cm to 4.86 cm, the Kocayaşar variety from 0.90 cm to 5.57 cm, and the Zeybek variety from 1.26 cm to 6.93 cm, with the highest root length observed in the Zeybek variety at 30°C. Increasing salt doses negatively affected both shoot and root lengths, whereas higher temperatures had a positive impact on these parameters. Successful plant growth under stress conditions largely depends on effective shoot and root development. Following successful seed germination, a robust seedling stage is crucial, and identifying the optimal temperatures for different species under stress conditions is essential. Osmotic and ionic effects are considered the dominant factors that inhibit seed germination responses under salt stress (Debez et al., 2004). The detrimental effects of salinity generally decrease at optimal temperatures (Khan et al., 2002). Studies have reported that interactive effects of salinity and temperature linearly reduce root and shoot lengths in Lolium species (Marcum and Pessarakli, 2013; Guo et al., 2020). Another study highlighted that shoot length is more severely affected than root length under stress conditions, and tetraploid Italian ryegrass exhibited better average root and shoot lengths compared to diploid varieties at varying temperature and salinity levels (Özkan et al. 2022).

3.4. Shoot Fresh and Dry Weights

In terms of shoot fresh and dry weights, variety, salt dose, variety x salt dose, variety x temperature, and variety x salt dose x temperature interaction were found to be significant at the 0.01 level.

As seen in Table 1, shoot fresh weight ranged from 31.40 mg to 64.60 mg for the İlkadım variety, from 29.27 mg to 66.43 mg for the Kocayaşar variety, and from 50.33 mg to 78.43 mg for the Zeybek variety. The highest shoot fresh weight was observed in the Zeybek variety at 20 °C. For shoot dry weight, the İlkadım variety ranged from 3.70 mg to 6.93 mg, the Kocayaşar variety from 2.50 mg to 7.17 mg, and the Zeybek variety from 5.23 mg to 8.87 mg (Table 1). The highest shoot dry weight was also observed in the Zeybek variety at 20 °C. Like shoot fresh and dry weights, stress effects were tolerable with the provision of optimal temperature. Increasing temperatures and salinity levels reduced the fresh weight in all varieties. The findings are consistent with previous studies. Özkan et al. (2022) noted that increasing temperatures and salinity levels decreased fresh weight, and that tetraploid varieties had higher fresh weights compared to diploid varieties. Other similar studies have reported that increasing salinity negatively affects both fresh and dry weights. Zabihi-e-Mahmoodabad et al. (2011) reported a decrease in both shoot and root fresh and dry weights with increasing salinity, and this feature was noted as a key indicator of salinity tolerance. Additionally, Hussein et al. (2007) found a negative relationship between vegetative growth parameters and increasing salinity.

Osmotic and ionic effects are generally accepted as the dominant factors hindering seed germination behavior under salt stress (Debez et al., 2004). Various studies have shown that germination responses are significantly affected by salinity and temperature. Salt stress includes osmotic and ionic stresses, leading to suppressed growth. The harmful effects continue as decreased plant survival or yield reduction. Conversely, one study indicated that there was no significant difference in germination percentage of Italian ryegrass between four different temperatures, and temperature alone was not a limiting factor in the absence of salinity if soil moisture was present (Lin et al., 2018). Özkan et al (2022) stated that the optimal temperature for seed germination of Italian ryegrass is between 20-30 °C.

The correlation graph evaluating the interaction of variety, salt, and temperature applications on the germination parameters of Italian ryegrass seeds supports the findings (Figure 1). Although germination rates vary by variety, the germination parameters are positively affected under conditions where low salt and high-temperature applications are combined. Additionally, the effect of three different salt doses (control, 5, and 10 EC) and three different temperatures (15, 20, and 30 °C) on germination parameters is supported by PCA analyses. According to the calculated

PCA analysis, the graphs explain 86% of the applications (Component 1 73.5%; Component 2 12.9%). When the obtained findings are examined, it is observed that there is a linear relationship between temperature and the germination of Italian ryegrass seeds. As the temperature increases, the tendency to form roots and shoots also increases (Figure 2).

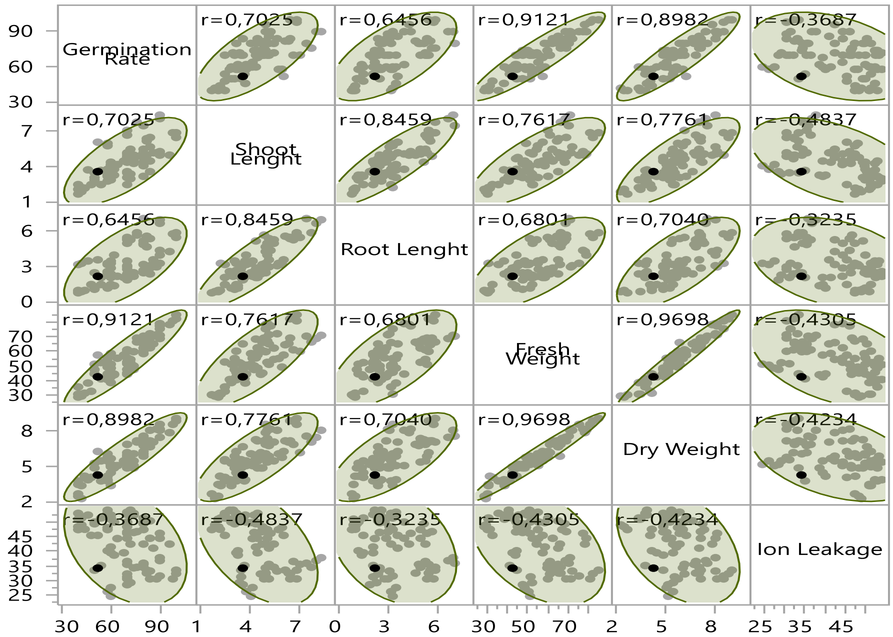

Figure 1. Scatterplot matrix and correlation of the germination parameters of Italian ryegrass with different salt and temperature treatments.

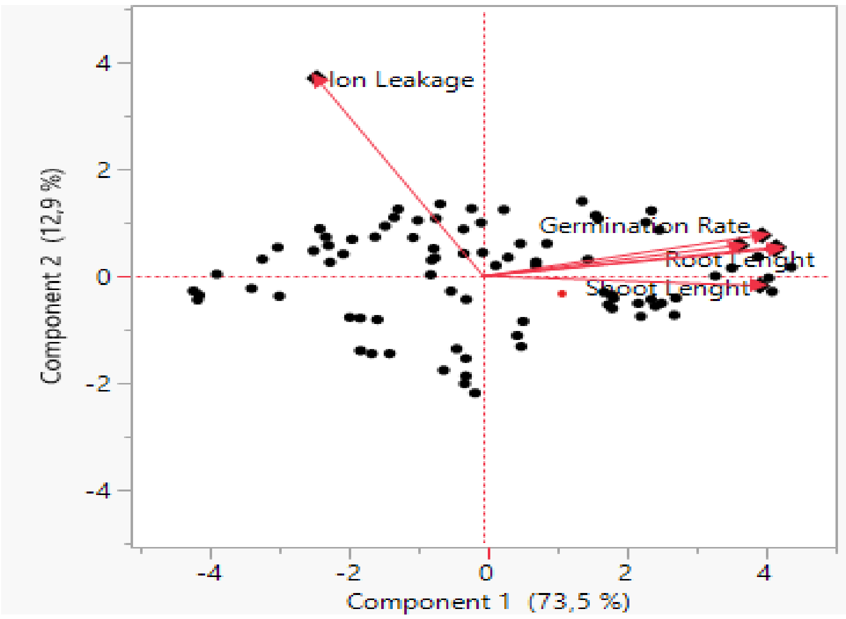

Figure 2. Principal Component Analysis (PCA) between germination parameters of Italian ryegrass with different salt and temperature treatments.

When looking at the ion leakage graph, it is seen that salt applications are positioned in the opposite direction to germination parameters. As the salt application in the environment increases, ion leakage also increases (Figure 2). The PCA graph evaluating the interaction of variety, salt, and temperature applications on the germination parameters of Italian ryegrass seeds also supports the findings obtained. Although germination rates vary by variety, the germination parameters are positively affected under conditions where low salt and high-temperature applications are combined. Under high-temperature conditions, similar results to the control were obtained when low salt doses were applied.

4. Conclusion

Salinity and high temperatures are significant abiotic stress factors that negatively impact plant growth and productivity, thereby limiting agricultural production. This study investigated the effects of different salt and temperature treatments on the germination parameters of Italian ryegrass seeds. The results indicated that salinity significantly inhibited the germination of Italian ryegrass seeds. As the salt concentration increased, a notable decrease in germination rate was observed. Increased salt stress also led to higher ion leakage, accompanied by ion toxicity. This is believed to obstruct water uptake in seeds, limiting germination as the environmental salt concentration rises. Similar results were obtained across the three varieties used in the study. Additionally, it was observed that the damage caused by salt stress decreased with increasing temperature. This study serves as a foundational investigation, and the grass species used here can be applied both as animal feed and for landscaping purposes. In the context of sustainable agriculture, responsible production and consumption, and addressing global climate issues, this research is valuable for breeding varieties that are resistant to salinity and temperature stress.

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.Ş.	S.U.
C	70	15	15
D	50	50	
S		30	70
DCP	60	30	10
DAI	60	30	10
L	30	40	30
W	30	40	30
CR	20	60	20
SR	20	60	20
PM	15	15	70
FA	10	10	80

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. 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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