

## The Effect of Salinity Stress on the Germination and Early Growth Parameters of Selected Ryegrass Species

## Şükrü Sezgi ÖZKAN <sup>1\*10</sup>, Esra ÇETİNKAYA ÖZKAN <sup>210</sup>

<sup>1</sup> Ege University, Faculty of Agriculture, Department of Field Crops, Izmir

<sup>2</sup> Firat University, Faculty of Architecture, Department of Landscape Architecture, Elazig

\*Corresponding author: sukru.sezgi.ozkan@ege.edu.tr

#### Abstract

Ryegrass (Lolium sp.) species are widely utilized in turfgrass areas worldwide due to their rapid establishment, aesthetic appeal, and adaptability to various environmental conditions. Salt stress is a significant environmental factor that adversely affects the growth and development of turfgrass species; this situation particularly threatens the health and performance of turfgrasses during the germination phase. The objective of the study was to evaluate the germination and early growth parameters of four different ryegrass species (annual ryegrass "Lolium multiflorum" cv. Axcella, diploid perennial ryegrass "Lolium perenne" cv. Sun, tetraploid perennial ryegrass "Lolium perenne" cv. Tetragreen, and intermediate ryegrass "Lolium hybridum" cv. TransAm) under different salinity stress levels (0, 2.5, 5.0, 7.5, 10.0, 12.5 g L<sup>-1</sup> sodium chloride-NaCl). In the study, germination rate (%), shoot and root length (cm), shoot and root fresh weight (mg), vigor index, salt tolerance index (%), and thousand-seed weight (g) parameters were determined. According to the results, salinity stress levels had significant effects on all treatments. The selected ryegrass species demonstrated better growth under a salinity stress level of 2.5 g L<sup>-1</sup> than the control. However, as the salt concentration increased, significant declines were observed in both germination and early growth, starting at 7.5 g L<sup>-1</sup>. Among the species, annual ryegrass cv. Axcella exhibited better performance under salinity stress. Additionally, the correlation coefficient results indicated a positive and significant correlation among all parameters, except for those involving the thousand-seed weight.

#### **Research Article**

Article History	
Received	:24.08.2024
Accepted	:28.09.2024

#### Keywords

Turfgrass germination salinity thousand-seed weight correlation

## 1. Introduction

Salinity stress has become a significant issue in turfgrass management, as in many other fields (Huang et al., 2014). Improper agricultural or landscape irrigation practices, particularly in arid and semi-arid regions with poor natural drainage conditions, can lead to salinity problems (Kara et al., 2005). In efforts to conserve freshwater resources, recycled, waste, or reclaimed non-potable water may become an important irrigation source for turfgrass in these regions (Marcum, 2006). Salinity is expressed in decisiemens (dS m<sup>-1</sup>). Soil salinity exceeding 4 dS m<sup>-1</sup> causes plants to suffer damage, and at salinity levels above 15 dS m<sup>-1</sup>, only a few turfgrass species can thrive (Karaguzel, 2007). Salts present in the germination medium increase the osmotic pressure of the environment, preventing seeds from absorbing water or negatively affecting germination due to the toxic effects of ions such as Na<sup>+</sup> and Cl<sup>-</sup> (Essa, 2002).

Species of the genus ryegrass (Lolium sp.), in addition to being the most widely used cool- $(C_3)$ turfgrasses globally, season are commonly employed for overseeding warmturfgrasses, especially season  $(C_4)$ in Mediterranean and similar climates. to mitigate winter discoloration (Christians, 2011). The ryegrass genus includes perennial ryegrass (Lolium perenne L.), annual ryegrass (Lolium multiflorum Lam.), and their hybrid, intermediate ryegrass (Lolium hybridum), all of which can be used in turfgrass areas. Of these species, perennial ryegrass is commonly utilized in modern turf systems due to its superior quality (Turgeon, 2005). Perennial ryegrass (Lolium perenne L.), with its diploid chromosome structure (2n=2x=14), is widely used in applications ranging from home lawns to sports fields due to its characteristics, such as rapid establishment, high wear tolerance, the ability to withstand low mowing heights, and medium-fine leaf texture (Cereti et al., 2010). Through breeding programs, tetraploid perennial ryegrass varieties (Lolium perenne L., 2n=4x=28) have been developed and introduced to the market, featuring superior germination and vigor compared to diploid varieties. These tetraploid varieties also possess excellent colour and quality parameters and the ability to provide a uniform spring transition, especially in overseeding applications for warm-season turfgrasses (Richardson et al., 2007; Summerford et al., 2009).

Perennial ryegrass is moderately tolerant to salinity and can tolerate soil electrical conductivity (EC) ranging from 4 to 8 dS m<sup>-1</sup> (Harivandi et al., 1992). Annual and hybrid rvegrass species are more salt-sensitive. tolerating soil EC between 3 and 5 dS m<sup>-1</sup> (Marcum, 2006). Nizam (2011) reported that in perennial ryegrass, germination decreased by 79.3% at 24 dS m<sup>-1</sup> compared to the control, and both root and shoot fresh and dry weights significantly decreased above 8 dS m<sup>-1</sup>. Studies by Kusvuran et al. (2015) and Tatar et al. (2018), which examined the effects of different salt concentrations on the germination and seedling development of perennial ryegrass varieties, found that as salt concentrations increased, germination rate, shoot length, root length, shoot fresh weight, root fresh weight, and salt tolerance index decreased. Many researchers investigating the effects of salinity on turfgrass germination have expressed that increasing salt concentrations negatively affect germination and seedling growth (Qian et al., 2007; Dai et al., 2009).

Although numerous studies exist on the salinity tolerance range of diploid varieties of perennial ryegrass, research on the salinity tolerance of seeds from tetraploid perennial ryegrass and other ryegrass species, which have become increasingly popular in turfgrass areas in recent years, is limited. This study aims to determine the salinity tolerance of some widely used worldwide ryegrass species during germination and the early growth stage.

#### 2. Materials and Methods

This study was conducted in 2021 at the Seed Laboratory of Department of Field Crops, Faculty of Agriculture, Ege University, to determine the effects of different salinity stress levels on the germination and early growth parameters of selected ryegrass (*Lolium* sp.) species. Four different ryegrass species (annual ryegrass "*Lolium multiflorum*" cv. Axcella, diploid perennial ryegrass "*Lolium perenne*" cv. Sun, tetraploid perennial ryegrass "*Lolium perenne*" cv. Tetragreen, and intermediate ryegrass "*Lolium perenne x Lolium multiflorum - Lolium hybridum*" cv. TransAm) were used as plant material in the experiment.

The experimental design was a randomized plot design with four replications. The solutions with six different salinity levels (0, 2.5, 5.0, 7.5, 10.0, 12.5 g L<sup>-1</sup>) for salinity stress were adjusted using sodium chloride (NaCl). Distilled water was used as the control solution.

Firstly, thousand-seed weights of selected ryegrass species were calculated based on the weight of four subsamples of 100 seeds (ISTA, 2018). Then, seeds were initially treated with a 1.0% solution of sodium hypochlorite (NaClO) for 5 min for surface sterilization. Residual chlorine was eliminated by thorough washing of seeds with distilled water.

For the germination tests, 96 glass petri dishes with a diameter of 15 cm were used. Fifty seeds were placed on double-layered Whatman's No.1 filter papers inside the petri dishes. Ten milliliters of solution containing different doses of salt (NaCl) were added to each petri dish (Delatorre-Herrera and Pinto, 2009), and the papers were replaced every two days to prevent the accumulation of salts (Rehman et al., 1997). To prevent evaporation, petri dishes were put into locked transparent plastic bags (Kaya et al., 2005). Salt tolerance tests during the germination period were conducted in a growth chamber with a fixed temperature of  $20\pm1^{\circ}$ C under dark conditions for 10 days (ISTA, 2018).

Daily observations were conducted for the evaluated parameters, and seeds were considered germinated once root lengths exceeded 2 mm (ISTA, 2018). The germinated seeds were counted and converted to percentages. Shoot/root lengths and shoot/root fresh weights were measured on ten seedlings at the end of the 10th day. Root and shoot lengths of the selected seedlings were measured using a millimeter ruler. The vigor index (Hu et al., 2005) and salt tolerance index (Budakli Carpici et al., 2009) were calculated using the specified formulas:

Vigor Index = [Germination percentage x (root length + shoot length)]

# Salt Tolerance Index = (Total fresh weight at $S_x$ / Total fresh weight at $S_0$ ) x 100 $S_x$ : Salt concentration, $S_0$ : Control

All data were statistically analyzed using analysis of variance (ANOVA) in the Statistical Analysis System (SAS Institute, 2012). Differences with a probability of 0.05 or less were considered significant. If ANOVA identified significant differences between treatment means, the least significant difference (LSD) test was conducted to distinguish them (Johnson and Bhattacharyya, 2019). Additionally, Pearson's correlation analysis was applied following Crawford (2006) to examine the relationships between parameters under salinity stress.

#### **3. Results and Discussion 3.1. Thousand-seed weight**

Following ISTA (2018) guidelines, the thousand-seed count was conducted in four replicates for each ryegrass species, and the thousand-seed weight was calculated accordingly (Figure 1). Among the four ryegrass species examined, the highest average thousand-seed weight was recorded in intermediate ryegrass cv. TransAm seeds at 3.20 g, while the lowest was found in diploid perennial ryegrass cv. Sun seeds at 1.78 g.



#### **3.2. Germination rate**

It was determined that germination rates differed according to species and salt doses, and these differences also had significant effects on the interaction (Table 1). Therefore, when interaction values are examined, it is noted that germination rates range between 36.00 and 98.50%. The highest germination rate was obtained from tetraploid perennial ryegrass cv. Tetragreen seeds at the control dose. Conversely, the lowest germination rates were recorded at 12.5 g  $L^{-1}$  dose, with 36.00 and 36.50% for tetraploid perennial ryegrass cv. Tetragreen and intermediate ryegrass cv. TransAm seeds, respectively. Regarding the examined, the species highest average germination rate was 83.00% for tetraploid perennial ryegrass cv. Tetragreen and 81.17% for diploid perennial ryegrass cv. Sun seeds, while the lowest average value was 75.00% for intermediate ryegrass cv. TransAm. The significance of salt doses suggested a decreasing trend in germination rates as the dose increases. The highest average germination rate was observed in the control

treatment (96.75%), while the lowest was found at the 12.5 g  $L^{-1}$  salt dose (40.38%).

Germination is one of the most critical stages of a plant's life cycle, and plants are susceptible to salt stress during this period (Salisbury and Ross, 1992). Typically, the highest germination rates occur in salt-free conditions, with increasing salt concentration negatively affecting germination and seedling development (Shiade and Boelt, 2020; Tenikecier and Ates, 2022; Faraj et al., 2023). This result is thought to be due to the toxic effect of increased salt ions and the adverse impact of osmotic pressure on seed water uptake (Liu et al., 2023). Numerous researchers have reported that responses to salt concentrations vary significantly among plant species and varieties, with an overall decrease in germination rates as salt concentration increases (Kokten et al., 2010; Karakoy et al., 2012; Zhang et al., 2012; Kusvuran et al., 2014a; Kusvuran et al., 2014b; Demiroglu Topcu et al., 2016; Borawska-Jarmulowicz et al., 2017; Ceritoglu et al., 2020; Tod et al., 2021; Ozkan et al., 2022; Yesil et al., 2022).

# Özkan and Çetinkaya Özkan

<b>Table 1.</b> Effect of salinity stress on germination and early growth parameters of selected ryegrass spec
--

Solinity			Species		
Lovols	Annual	Diploid	Fetraploid perennia	l Intermediate	
$(\sigma \mathbf{L}^{-1})$	ryegrass	perennial ryegrass	ryegrass	ryegrass	Mean
(g 12 )	cv. Axcella	cv. Sun	cv. Tetragreen	cv. TransAm	
		Germination Rat	e (%)		
0.0	96.00 ab	96.50 ab	98.50 a	96.00 ab	96.75 a
2.5	88.50 cd	95.00 ab	96.50 ab	94.00 ab	93.50 b
5.0	87.00 d	92.50 bc	95.00 ab	85.00 de	89.88 c
7.5	86.50 d	88.00 cd	93.50 b	81.00 ef	87.25 d
10.0	65.00 h	72.50 g	78.50 f	57.50 i	68.38 e
12.5	46.50 j	42.50 j	36.00 k	36.50 k	40.38 f
Mean	/8.25 b	81.1/a	83.00 a	/5.00 c	/9.35
C.V.=4.04%		LSD (0.05) S: 1.84 <sup>2</sup>	** L: 2.26**	S x L: 4.51**	
	10.461	Shoot Lenght (	cm)	0.02.1	0.27
0.0	10.46 DC	7.41 gn 7.40 ab	9.98 cd	9.23 de	9.27 a
2.5	11.40 a	7.40 gli 7.25 ah	10.85 ab	0.51 el 7.44 ch	9.30 a 9.67 h
3.0 7.5	10.49 bc	7.55 gli 5.80 ;	9.40 d 8.10 fc	7.44 gii 5.27 ii	0.07 U 7 15 o
7.5	9.24 de	2 08 1-1	6.10 lg	2.57 IJ 2.601	1.15 C
12.5	4.84 ;	2.18 m	4.09 JK	1.03 m	4.77u
Mean	4.04 J 8.89 a	5 70 c	7.50 h	6.01 c	2.74 0
C V -8 56%	0.07 u	LSD (0.05) S: 0.35**	* I · 0 42**	S x I · 0.85**	7.05
0.1.0.0070		Boot Length (c	m)	5 A E. 0.05	
0.0	7 95 a	4 35 f-h	7 56 a	5 64 h-d	638 a
2.5	7.55 a	4.59 e-h	6.11 b	5.04 d d	5.82 h
5.0	7.50 a	4 54 e-h	5.99 bc	4.25 g-i	5.57 b
7.5	5.40 b-e	3.90 hi	5.16 c-f	3.41 i	4.47 c
10.0	4.83 d-g	2.39 i	3.83 hi	1.96 i-1	3.25 d
12.5	2.09 jk	1.25 kl	1.94 j-1	1.181	1.61 e
Mean	5.89 <sup>°</sup> a	3.50 c	5.10 b	3.58 c	4.52
C.V.=13.63%		LSD (0.05) S: 0.35**	* L: 0.43**	S x L: 0.87**	
		Shoot Fresh Weigh	nt (mg)		
0.0	9.67 c	7.00 e	10.75 b	9.24 c	9.17 b
2.5	12.12 a	8.02 d	12.24 a	6.90 e	9.82 a
5.0	10.86 b	6.84 e	11.21 b	5.91 f	8.71 c
7.5	8.39 d	5.47 fg	7.88 d	5.33 fg	6.77 d
10.0	5.92 f	4.23 h	4.46 h	4.50 h	4.78 e
12.5	5.20 g	3.13 i	1.27 k	2.00 j	2.90 f
Mean	8.69 a	5.78 c	7.97 b	5.65 c	7.03
C.V.=6.34%		LSD (0.05) S: 0.26**	L: 0.31**	S x L: 0.63**	
		Root Fresh Weigh	t (mg)		
0.0	1.66 a	0.82 fg	1.59 ab	1.26 c	1.33 a
2.5	1.60 ab	0.99 d-f	1.42 bc	1.06 d	1.27 a
5.0	1.54 ab	0.95 d-f	1.28 c	0.92 d-g	1.17 b
/.5	1.01 de	0.76 g	1.04 d	0.76 g	0.89 c
10.0	0.89 d-g	0.22 ;	0.84 e-g	0.40 m	0.67 d
12.3 Moon	1.21 0	0.331	0.49 III 1.11 h	0.511	0.42 6
C V -13 97%	1.21 a	LSD (0.05) S: 0.08**	* I · 0 09**	S v I · 0 10**	0.90
C. V.=13.7770		Vigor Index	L. 0.07	5 X L. 0.17	
0.0	1769 3 a	1134.7 hi	1727.2 ah	1426.2 p	15143 a
2.5	1683 3 a-c	1139.6 g-i	1636.7  bc	1420.2 C	1432.6 h
5.0	1565.5 cd	1101.2 ji	1460.5 de	993.7 i	1280.2 c
7.5	1263.5 fg	862.6 k	1239.1 f-h	710.11	1018.8 d
10.0	755.0 kl	460.5 m	668.21	320.5 n	551.0 e
12.5	322.7 n	145.0 o	142.0 o	113.5 0	180.8 f
Mean	1226.5 a	807.3 c	1145.6 b	805.8 c	996.3
C.V.=8.86%		LSD (0.05) S: 50.7**	L: 62.1**	S x L: 124.2**	
		Salt Tolerance Ind	lex (%)		
0.0	100.00 c	100.00 c	100.00 c	100.00 c	100.00 b
2.5	121.35 a	115.26 ab	110.71 b	75.85 ef	105.79 a
5.0	109.47 b	99.65 c	101.21 c	65.07 g	93.85 c
7.5	83.00 d	79.72 de	72.28 f	58.01 h	73.26 d
10.0	60.13 gh	61.15 gh	42.95 j	46.65 ij	52.72 e
12.5	50.98 i	44.25 ij	14.271	22.03 k	32.88 f
Mean	87.49 a	83.34 b	73.57 c	61.27 d	76.42
C V =6.53%		LSD (0.05) S: 2.87*	* L:3.51**	S x L: 7.03**	

ns: non-significant, S: species, L: salinity level, \*: significant at 0.05 level, \*\*: significant at 0.01 level

## 3.3. Shoot length

An analysis of the effects of different salinity levels on shoot length in selected statistically ryegrass species revealed significant differences (Table 1). The highest shoot length (11.48 cm) was observed in the 2.5 g  $L^{-1}$  treatment with annual ryegrass cv. Axcella seeds, while the lowest (1.93 cm) occurred in the 10.0 g L<sup>-1</sup> treatment with intermediate ryegrass cv. TransAm seeds. Shoot lengths decreased as salinity increased. During germination, shoot length is a critical indicator of seedling vigor and overall plant health. Environmental stresses such as salinity can significantly reduce shoot elongation, directly affecting plant establishment and (Salisbury growth and Ross, 1992). Researchers have frequently highlighted the negative impact of salt stress on shoot development plants (Borawskain Jarmulowicz et al., 2017; Demiroglu Topcu and Ozkan, 2020; Ertekin, 2021; Javaid et al., 2022; Tang et al., 2022). Additionally, salinity is generally reported to affect aboveground plant parts more severely than belowground parts (Pawlowicz et al., 2018).

#### 3.4. Root length

Root lengths varied depending on both species and salinity levels, with these differences also significantly impacting the interaction (Table 1). An examination of the interaction values reveals that shoot lengths ranged from 1.18 to 7.95 cm. The longest root length (7.95 cm) was recorded in the control treatment with seeds from annual ryegrass cv. Axcella. Conversely, the shortest root length (1.18 cm) was observed in the 12.5 g  $L^{-1}$ treatment with seeds from intermediate ryegrass cv. TransAm. Root development is a crucial factor in salt tolerance, with optimal root growth occurring when there is no salt stress during germination. High levels of Na<sup>+</sup> ions increase osmotic pressure in favor of soil water and induce ion toxicity, preventing the seed from absorbing water. This condition hinders plant growth and development (Nizam, 2011). The responses of plant species to salt concentrations differ considerably, and a reduction in root length with increasing salt

concentrations has been reported by many researchers (Muscolo et al., 2003; Nizam, 2011; Kusvuran et al., 2015; Borawska-Jarmulowicz et al., 2017; Xie et al., 2021; Yesil et al., 2022). Reductions in root development due to salt stress are known to result from decreased water uptake by the plant (Liu et al. 2023). In light of this information, root length emerges as a key parameter that can be used to select salt-tolerant genotypes (Khan et al., 2003).

#### 3.5. Shoot fresh weight

Data revealed that different salinity levels had statistically significant effects on the shoot fresh weights of ryegrass species (Table 1). In the study, the highest shoot fresh weight was recorded for tetraploid perennial ryegrass cv. Tetragreen and annual ryegrass cv. Axcella seeds, with 12.24 mg and 12.12 mg at the 2.5 g L<sup>-1</sup> salt dose, respectively. The lowest shoot fresh weight was recorded for tetraploid perennial ryegrass cv. Tetragreen seeds, with 1.27 mg at the 12.5 g  $L^{-1}$  salt dose. A serious decrease in shoot fresh weight was observed across the different ryegrass species as salt concentration increased. The shoot fresh weight value of 9.82 mg obtained at the 2.5 g  $L^{-1}$  salt dose decreased to 2.90 mg at the 12.5 g  $L^{-1}$  dose. Salinity is known not only to exert osmotic stress on plants but also to cause damage due to the toxic effects of ions. In many plant species, significant growth retardation is observed as ion concentrations, particularly Na<sup>+</sup> and Cl<sup>-</sup>, increase under saline conditions. Additionally, NaCl reduces the rate of photosynthesis and increases the rate of respiration, resulting in a decline in net photosynthesis and subsequently hindering plant growth (Salisbury and Ross, 1992). Demiroglu Topcu et al. (2016) reported that high salt concentrations increase the osmotic pressure in the environment, thereby reducing water absorption and leading to a decrease in fresh weight. Similar studies with grass species have demonstrated a reduction in shoot fresh weight with increasing salt concentrations (Tatar et al., 2018; Yilmaz and Kisakurek, 2018; Turk and Alagoz, 2020; Tod et al., 2021; Ertekin. 2021; Ozkan et al.. 2022).

Furthermore, it is reported that significant differences exist between varieties in terms of shoot fresh weight, and that salt-tolerant varieties selected based on shoot development should be cultivated in areas facing salinity problems (Liu et al., 2023).

#### 3.6. Root fresh weight

The effects of different salinity levels on the root fresh weights of ryegrass species were statistically significant (Table 1). The highest root fresh weight (1.66 mg) was observed in annual ryegrass cv. Axcella seeds in the control treatment, whereas the lowest values were recorded for intermediate ryegrass cv. TransAm and diploid perennial ryegrass cv. Sun seeds, with 0.31 and 0.33 mg at the 12.5 g L<sup>-1</sup> dose, respectively. Similar to shoot fresh weight, root fresh weight decreased as salt concentration increased. The highest average root fresh weight (1.33 mg) was obtained from the control, while the lowest (0.42 mg) was observed at the 12.5 g  $L^{-1}$  salt dose. Among the species, annual ryegrass cv. Axcella had the highest average root fresh weight at 1.21 mg, while the lowest average values were recorded for diploid perennial ryegrass cv. Sun (0.73 mg) and intermediate ryegrass cv. TransAm (0.79 mg). It is known that salinity in growth environments impacts root fresh weight, as salt stress limits water uptake, leading to reduced fresh weight (Salisbury and Ross, 1992; Munns, 2002). Previous studies have noted that perennial ryegrass varieties respond differently to salt concentrations, though root fresh weight decreases across all varieties as salt levels rise (Nizam, 2011; Kusvuran et al., 2015; Tatar et al., 2018; Yilmaz and Kisakurek, 2018; Tod et al., 2021; Javaid et al., 2022; Tenikecier and Ates, 2022). Our findings confirmed that increasing salinity levels significantly reduced root fresh weights in the selected ryegrass species.

#### 3.7. Vigor index

The effects of different salinity levels on the vigor index of selected ryegrass species revealed statistically significant differences (Table 1). The highest vigor index value of 1769.3 was obtained from annual ryegrass cv. Axcella seeds in the control treatment. A

substantial decrease in vigor index was observed with increasing salt doses, with the lowest values recorded for intermediate ryegrass cv. TransAm, tetraploid perennial ryegrass cv. Tetragreen, and diploid perennial ryegrass cv. Sun, yielding indices of 113.5, 142.0, and 145.0, respectively. Significant reductions in vigor index values were noted as salinity level increased. The highest average vigor index value was recorded in the control application at 1514.3, while the lowest average value occurred at the 12.5 g L<sup>-1</sup> salt dose, at 180.8. Among the ryegrass species, the highest average vigor index value was found in annual ryegrass cv. Axcella seeds at 1226.5, whereas the lowest average values were recorded in intermediate ryegrass cv. TransAm (805.8) and diploid perennial ryegrass cv. Sun (807.3). Tatar et al. (2018) reported a decrease in the vigor index with increasing salt concentration on perennial ryegrass, with the lowest vigor index observed at the highest salt concentration. Ozturk et al. (2018) applied salt stress to tall fescue seeds pretreated with polyethylene glycol and observed the highest vigor index in the control group. Similarly, Shiade and Boelt (2020) and Yesil et al. (2022) noted in their studies that salinity stress negatively affected the vigor index. Our study aligns with these findings, confirming that increasing salinity level leads to declines in vigor index values.

# 3.8. Salt tolerance index

Statistical analysis results regarding the salt tolerance index indicated significant differences at the 0.05 level among ryegrass species, salinity level, and their interaction (Table 1). Although the salt tolerance index tended to decrease after a 2.5 g L<sup>-1</sup> salt dose, the varying responses of the examined ryegrass species to salinity levels contributed to the significance of the interaction. The study showed that the salt tolerance index ranged from 75.85 to 121.35% under the 2.5 g  $L^{-1}$  salt dose, while it varied from 14.27 to 50.98% under the 12.5 g  $L^{-1}$  salt dose, indicating that the response of ryegrass species to salinity can be quite different. The highest salt tolerance index of 121.35% was recorded for seeds of annual ryegrass cv. Axcella at the 2.5 g L<sup>-1</sup> salt dose, whereas the lowest value of 14.27% was obtained from tetraploid perennial ryegrass cv. Tetragreen at the 12.5 g L<sup>-1</sup> salt dose. The study revealed a significant decrease in salt tolerance index values with increasing salinity levels, with the highest average value of 105.79% recorded at the 2.5 g L<sup>-1</sup> salt dose, while the lowest average value was 32.88% at the 12.5 g L<sup>-1</sup> salt dose. Among ryegrass species, the highest average salt tolerance index value was found to be 87.49% in seeds of annual ryegrass cv. Axcella, while the lowest average value of 61.27% was recorded in seeds of intermediate ryegrass cv. TransAm.

The ability of plants to maintain growth and development in environments with elevated salt levels is defined as salt tolerance (Liu et al., 2023). Under saline conditions, plants typically accumulate Na<sup>+</sup> and Cl<sup>-</sup> ions in their roots, stems, and leaves. This ion accumulation in various plant organs can impede growth and significantly impact salt tolerance (Salisbury and Ross, 1992; Munns, 2002). Researchers have indicated that the salt tolerance index decreases with increasing salinity levels (Kusvuran et al., 2015; Tatar et al., 2018; Turk and Alagoz, 2020; Xie et al., 2021; Tod et al.,

2021; Tenikecier and Ates, 2022). In our study, a marked decrease in salt tolerance index values was observed with rising salinity levels. However, the negative impact of increasing salinity on annual ryegrass cv. Axcella and diploid perennial ryegrass cv. Sun seeds were significantly less pronounced compared to other species.

#### 3.9. Correlation analysis

The correlation coefficient of the studied parameters was analyzed by Pearson's correlation. The Pearson correlation matrix illustrates the relationships between various parameters, with correlation measured coefficients ranging from -1.00 to 1.00. A positive correlation is shown in red, while a negative correlation is shown in blue, with darker colors indicating stronger correlations. The correlation among the studied parameters is presented in Figure 2. Thousand-seed weight showed weak correlations with most other parameters, including a negative relationship salt tolerance index (-0.28) and with germination rate (-0.07), and near-zero correlations with shoot length (0.04), root length (0.04), shoot fresh weight (0.02), root fresh weight (0.08), and vigor index (0.03), indicating little or no linear relationships.



Figure 2. The correlation coefficient of the studied parameters

TSW: thousand-seed weight, GR: germination rate, SL: shoot length, RL: root length, SFW: shoot fresh weight, RFW: root fresh weight, VI: vigor index, STI: salt tolerance index

In contrast, germination rate exhibited strong positive correlations with growth parameters such as shoot length (0.82), root length (0.76), shoot fresh weight (0.77), root fresh weight (0.73), vigor index (0.88), and salt tolerance index (0.84), suggesting that higher germination rates were associated with increased growth performance. Similarly, shoot length, root length, shoot fresh weight, and root fresh weight were highly correlated with one another, indicating that these growth parameters tended to increase together. Notably, the salt tolerance index showed strong positive correlations with growth variables but a negative relationship with thousand-seed weight, suggesting that larger seed weights might not have been beneficial under salinity stress conditions.

#### 4. Conclusions

In every city, particularly in turf areas, which are fundamental to landscape design, the plant materials must be resistant to environmental factors in the respective ecology to ensure the sustainability and economic viability of the established facilities. It is known that the responses of plant species to stress conditions vary significantly. To successfully cultivate suitable plants under specific conditions, it is essential to understand these responses and adjust the environment to meet the plants' needs.

In this study, the adaptation of some ryegrass species, commonly used in turf areas, to saline conditions was tested under laboratory conditions. The results indicated that the salt concentrations applied to the selected ryegrass species had significant effects on the studied parameters. Moreover, the correlation coefficient results revealed a positive, significant correlation among all combinations, except for those involving the thousand-seed weight. The selected ryegrass species examined exhibited better growth at a salinity stress level of 2.5 g L<sup>-1</sup> compared to the control. However, with increasing salt concentration. significant declines were observed in both germination and early growth, starting at 7.5 g L<sup>-1</sup>. According to the findings, annual ryegrass cv. Axcella was

performed better under salinity stress. Nevertheless, considering that around 200 parameters can be used to evaluate the effects of salt stress, more parameters should be examined for more accurate conclusions. Expanding the study to include additional species and varieties, as well as testing them under field conditions, would yield more reliable results and provide valuable guidance for selecting species and varieties suitable for specific ecological conditions.

## **Declaration of Author Contributions**

The authors declare that they have contributed equally to the article. All authors declare that they have seen/read and approved the final version of the article ready for publication.

#### **Declaration of Conflicts of Interest**

All authors declare that there is no conflict of interest related to this article.

#### References

- Borawska-Jarmulowicz, B., Mastalerczuk, G., Maluszynska, Gozdowski, D., E., Szydlowska, A., 2017. The sensitivity of Lolium perenne and Poa pratensis to salinity and drought during the seed germination under different and photoperiod conditions. Zemdirbvste-Agriculture, 104(1): 71-78.
- Budakli Carpici, E., Celik, N., Bayram, G., 2009. Effects of salt stress on germination of some maize (*Zea mays L.*) cultivars. *African Journal of Biotechnology*, 8(19): 4918-4922.
- Cereti, C.F., Ruggeri, R., Rossini, F., 2010. Cool-season turfgrass species and cultivars: Response to simulated traffic in central Italy. *Italian Journal of Agronomy*, 5(1): 53-59.
- Ceritoglu, M., Erman, M., Yildiz., F., 2020. Effect of salinity on germination and some agro-morphological traits in chickpea seedlings. *ISPEC Journal of Agricultural Sciences*, 4(1): 82-96.

- Christians, N.E., 2011. Fundamentals of turfgrass management (fourth edition). Wiley, New York, NY.
- Crawford, S.L., 2006. Correlation and regression. *Circulation*, 114(19): 2083-2088.
- Dai, J., Huff, D.R., Schlossgerg, M.J., 2009. Salinity effects on seed germination and vegetative growth of greens-type *Poa annua* relative to other cool-season turfgrass species. *Crop Science*, 49(2): 696-703.
- Delatorre-Herrera, J., Pinto, M., 2009. osmotic Importance of ionic and components of salt stress on the germination of four quinua (Chenopodium quinoa Willd.) selections. Chilean Journal of Agricultural Research, 69(4): 477-485.
- Demiroglu Topcu, G., Celen, A.E., Kuru, E., Ozkan, S.S., 2016. A study on the effects of concentrations different NaCl on germination and early growing stage of tall *arundinacea*) fescue (Festuca and intermediate wheatgrass (Agropyron intermedium). Journal of Central Research Institute for Field Crops, 25(Special Issue-2): 219-224.
- Demiroglu Topcu, G., Ozkan, S.S., 2020. Effects of different salt sources and concentrations on germination parameters of barley (*Hordeum vulgare* L.) seeds. *ISPEC Journal of Agricultural Sciences*, 4(3): 456-467.
- Ertekin, I., 2021. Evaluation of some annual ryegrass cultivars in germination and early seedling stage under different salinity levels. *III. Balkan Agriculture Congress*, Congress Book, 29 August - 1 September, Edirne, s:363-369.
- Essa, T.A., 2002. Effect of salinity stresson growth and nutrient composition of threesoybean (*Glycine max* L. Merrill) cultivars. *Journal of Agronomy and Crop Science*, 188(2): 86-93.

- Faraj, A.H., Colak, E.S., Isik, D., 2023. Environmental factors influencing *Lolium temulentum* L. (Darnel ryegrass) seed germination. *Annali di Botanica (Roma)*, 13: 11-18.
- Harivandi, M.A, Butler, J.D., Wu, L., 1992. Salinity and turfgrass culture. *Turfgrass*, 32: 207-229.
- Hu, J., Zhu, Z.Y., Song, W.J., Wang, J.C., Hu, W.M., 2005. Effects of sand priming on germination and field performance in direct-sown rice (*Oryza sativa* L.). Seed Science and Technology, 33(1): 243-248.
- Huang, B., DaCosta, M., Jiang, Y., 2014. Research advances in mechanisms of turfgrass tolerance to abiotic stresses: from physiology to molecular biology. *Critical Reviews in Plant Sciences*, 33(2-3): 141-189.
- ISTA, 2018. International Rules for Seed Testing Book. International Seed Testing Association (ISTA) Press, Switzerland.
- Javaid, M.M., Mahmood, A., Alshaya, D.S., AlKahtani, M.D., Waheed, H., Wasaya, A., Khan, S.A., Naqve, M., Haider, I., Shahid, M.A., Nadeem, M.A., Azmat, S., Khan, B.A., Balal, R.M., Attia, K.A., Fiaz, S., 2022. Influence of environmental factors on seed germination and seedling characteristics of perennial ryegrass (Lolium perenne L.). Scientific Reports, 12(1): 9522.
- Johnson, R.A., Bhattacharyya, G.K., 2019. Statistics: principles and ethods (8th edition). Wiley & Sons Inc.
- Kara, E., Ekmekci, M., Tekin, M.A., 2005. The effect of salinity on plant growth. *Journal of Faculty of Agriculture, Ondokuz Mayıs University*, 20(3): 118-125.
- Karaguzel, O., 2007. Lawn and ground cover plants lecture notes (unpublished). Akdeniz University, Faculty of Agriculture, Department of Landscape Architecture, Antalya/Türkiye.

- Karakoy, T., Kokten, K., Toklu, F., 2012. Response of some chickpea (*Cicer arietinum* L.) genotypes to salt stress conditions. *International Journal of Food*, *Agriculture and Environment*, 10(3&4): 337-341.
- Kaya, M.D., Kaya, G., Kolsarici, O. 2005. Effects of NaCl concentration on germination and emergence of some *Brassica* species. *Journal of Agricultural Sciences*, 11(4): 448-452.
- Khan A.A., Rao, S.A., McNeilly, T., 2003. Assessment of salinity tolerance based upon seedling root growth response functions in maize (*Zea mays* L.). *Euphytica*, 131: 81-89.
- Kokten, K., Karakoy, T., Bakaoglu, A., Akcura, M., 2010. Determination of salinity tolerance of some lentil (*Lens culinaris* M.) varieties. *International Journal of Food*, *Agriculture and Environment*, 8(1): 140-143.
- Kusvuran, A., Nazli, R.I., Kusvuran, S., 2014a. Determination of salinity effects on seed germination in different red fescue (*Festuca rubra* L.) varieties. *Research Journal of Agricultural Sciences*, 7(1): 22-27.
- Kusvuran, A., Nazli, R.I., Kusvuran, S., 2014b. Salinity effects on seed germination in different tall fescue (*Festuca arundinaceae* Schreb.) varieties. *Research Journal of Agricultural Sciences*, 7(2): 8-12.
- Kusvuran, A., Nazli, R.I., Kusvuran, S., 2015. The effects of salinity on seed germination in perennial ryegrass (*Lolium perenne* L.) varieties. *Turkish Journal of Agricultural and Natural Sciences*, 2(1): 78-84.
- Liu, H., Todd, J.L., Luo, H., 2023. Turfgrass salinity stress and tolerance-A review. *Plants*, 12(4): 925.
- Marcum, K.B., 2006. Use of saline and nonpotable water in the turfgrass industry: Constraints and developments. *Agricultural Water Management*, 80(1-3): 132-146.

- Munns, R., 2002. Comparative physiology of salt and water stress. *Plant, Cell & Environment*, 25(2): 239-250.
- Muscolo, A., Panuccio, M.R., Sidari, M., 2003. Effects of salinity on growth, carbohydrate metabolism and nutritive properties of kikuyu grass (*Pennisetum clandestinum* Hochst). *Plant Science*, 164(6): 1103-1110.
- Nizam, I., 2011. Effects of salinity stress on water uptake, germination and early seedling growth of perennial ryegrass. *African Journal of Biotechnology*, 10(51): 10418-10424.
- Ozkan, U., Benlioglu, B., Telci Kahramanogullari, C., 2022. A comparison of germination responses on Italian ryegrass (diploid vs tetraploid) seeds to interactive effects of salinity and temperature. *Polish Journal of Environmental Studies*, 31(5): 4229-4237.
- Ozturk, Y., Tatar, N., Budakli Carpici, E., 2018. The effects of polyethylene glycol primings of tall fescue (*Festuca arundinacea* Schreb.) seeds on germination characters of seeds on salt stress conditions. *Journal of Agricultural Faculty of Uludag University*, 32(1): 141-149.
- Pawlowicz, I., Waskiewicz, A., Perlikowski, D., Rapacz, M., Ratajczak, D., Kosmala, A., 2018. Remodeling of chloroplast proteome under salinity affects salt tolerance of *Festuca arundinacea*. *Photosynthesis Research*, 137: 475-492.
- Qian, Y.L., Fu, J.M., Wilhelm, S.J., Christensen, D., Koski, A.J., 2007. Relative salinity tolerance of turf-type saltgrass selections. *HortScience*, 42(2): 205-209.
- Rehman, S., Harris, P.J.C., Bourne, W.F., Wilkin, J., 1997. The effect of sodium chloride on germination and the potassium and calcium content of acacia seeds. *Seed Science and Technology*, 25(1): 45-57.

- Richardson, M.D., Hignight, K.W., Walker, R.H., Rodgers, C.A., Rush, D., McCalla, J.H., Karcher, D.E., 2007. Meadow fescue and tetraploid perennial ryegrass-Two new species for overseeding dormant bermudagrass turf. *Crop Science*, 47(1): 83-90.
- Salisbury, F.B., Ross, C.W., 1992. Plant Physiology. Wadsworth Pub. Com. Inc., Belmont, California-USA.
- SAS Institute, 2012. SAS/STAT® software version 9.3 user's manual. Cary, NC: SAS Institute Inc.
- Shiade, S.R.G., Boelt, B., 2020. Seed germination and seedling growth parameters in nine tall fescue varieties under salinity stress. Acta Agriculturae Scandinavica, Section B-Soil & Plant Science, 70(6): 485-494.
- Summerford, J.A., Karcher, D.E., Richardson, M.D., Patton, A.J., Boyd, J.W., 2009. Cultural practice effects on the spring transition of overseeded meadow fescue and tetraploid perennial ryegrass sports fields. *International Turfgrass Society Research Journal*, 11: 501-510.
- Tang, J., Li, M., Mao, P., Jiang, Y., 2022. Effects of gamma-aminobutyric acid on seed germination, ion balance, and metabolic activity in perennial ryegrass under salinity stress. *Journal of Plant Growth Regulation*, 41(4): 1835-1844.
- Tatar, N., Ozturk, Y., Budakli Carpici, E., 2018. The effects of NaCl primings on germination characters of perennial ryegrass at different salt levels. *Turkish Journal of Agricultural and Natural Sciences*, 5(1): 28-33.

- Tenikecier, H., Ates, E., 2022. Impact of salinity on germination and seedling growth of four cool-season turfgrass species and cultivars. *Polish Journal of Environmental Studies*, 31(2): 1813-1821.
- Tod, M., Anghelus-Olenici, G., Balan, M., Andreoiu, A., Zevede, P., 2021. Effect of salinity on seed germination and seedling growing on some perennial grasses. *Journal* of Mountain Agriculture on the Balkans, 24(6): 277-290.
- Turgeon, A.J., 2005. Turfgrass Management (7th Edition). Prentice Hall, Englwood Cliffs, NJ.
- Turk, M., Alagoz, M., 2020. Effects of salt stress on the germination of tall fescue (*Festuca arundinacea* Schreb.) seeds. Journal of Agricultural Faculty of Bursa Uludag University, 34(2): 317-324.
- Xie, Y., Liu, X., Amee, M., Yu, H., Huang, Y.,
  Li, X., Chen, L., Fu, J., Sun, X., 2021.
  Evaluation of salt tolerance in Italian
  ryegrass at different developmental stages.
  Agronomy, 11(8): 1487.
- Yesil, P., Guzel, M., Sengur, S., 2022. Effects of different salt concentrations on the germination in some turfgrass varieties used in landscape applications. *Gumushane University Journal of Science and Technology*, 12(4): 1036-1045.
- Yilmaz, M.B., Kisakurek, S., 2018. Effect of salt stress on germination and early seedling stage of some perennial ryegrasses (*Lolium perenne* L.) cultivars. *Journal of Agricultural Faculty of Mustafa Kemal University*, 23(2): 204-217.
- Zhang, Q., Rue, K., Wang, S., 2012. Salinity effect on seed germination and growth of two warm-season native grass species. *Hortscience*, 47(4): 527-530.

	Özkan, Ş.S., Çetinkaya Özkan, E., 2025. The Effect of Salinity Stress on the Germination and
To Cite	Early Growth Parameters of Selected Ryegrass Species. ISPEC Journal of Agricultural
	<i>Sciences</i> , 9(1): 36-47.
	DOI: https://doi.org/10.5281/zenodo.14160787.