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The Influence of PEG-Induced Drought Stress on Seed Germination and Seedling Growth Traits of Tetraploid Annual Ryegrass Cultivars

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Abstract
Drought stress poses a significant challenge to global forage production, particularly under climate change conditions. Identifying cultivars with superior drought tolerance is essential for maintaining productivity in water-limited environments. The objective of the study was to evaluate the effects of polyethylene glycol (PEG)-induced drought stress on the germination and seedling growth traits of tetraploid annual ryegrass (*Lolium multiflorum* Lam.) cultivars. Four cultivars (Baqueano, Big Boss, Elif, and Medoacus) were exposed to seven osmotic potential levels (0, -0.2, -0.4, -0.6, -0.8, -1.0, and -1.2 MPa) under controlled conditions. Germination rate (%), mean germination time (day), shoot and root length (cm), shoot-to-root ratio, seedling fresh weight (mg), vigor index, and drought tolerance index (%) were determined. The results revealed significant reductions in germination and seedling growth traits with increasing drought stress. Among the cultivars, Baqueano and Big Boss cultivars exhibited superior drought tolerance, maintaining higher vigor and drought tolerance indices. An osmotic potential of -0.6 MPa reduced the drought tolerance index by up to 44.5%, with no germination observed at -1.2 MPa. Strong correlations were identified among germination rate, shoot and root length, vigor index, and drought tolerance index, highlighting their importance as indicators of drought resilience.

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1. Introduction

Climate change and environmental pollution have become pressing global challenges, profoundly impacting agricultural systems and plant productivity. Among various abiotic stressors, salinity and drought are two of the most significant threats to crop cultivation (Malhi et al., 2021). High soil salinity adversely affects plant development by reducing germination rates, impairing seedling establishment, and lowering yields. These effects are primarily due to decreased soil water potential and imbalances in the uptake and accumulation of essential nutrients within plant tissues (Jorjandi and Sharifi-Sirchi, 2012). Similarly, drought, defined as a period of prolonged water scarcity, poses significant challenges to plant growth, particularly in arid and semi-arid regions. Plants mitigate water stress through adaptive mechanisms, with osmoregulation playing a central role in enhancing drought tolerance (Khoyerdi et al., 2016).

Germination, the initial phase of a plant's life cycle, is a critical determinant of successful crop establishment and early growth. This stage is susceptible to abiotic stress conditions, including salinity and drought. Moreover, successful germination ensures the development of dense and uniform stands, which are essential for achieving optimal yields and maintaining crop quality (Benech-Arnold and Sánchez, 2004). However, research indicated that the seeds of most plant species germinate best under non-stress conditions, such as distilled water, whereas elevated levels of salinity or drought significantly inhibit germination and seedling growth (Budakli Carpici and Erdel, 2015; Demiroglu Topcu and Ozkan, 2016; Demiroglu Topcu et al., 2016; Demiroglu Topcu and Ozkan, 2020).

Annual ryegrass (*Lolium multiflorum* L.), a member of the Poeae tribe within the Poaceae family, is a significant forage species widely cultivated for livestock feed. Native to the Mediterranean region, annual ryegrass has adapted to diverse climates and is now extensively used for grazing, hay, and silage worldwide. Major production regions for annual ryegrass include temperate areas of Europe, the southern United States, parts of Australia, and New Zealand (Lopes et al., 2009). Cultivated for over a century, this diploid species is prized for its high forage quality, rapid establishment, and ability to thrive under cool-season conditions. In recent years, tetraploid varieties have gained popularity due to their higher forage yield and enhanced nutritional quality, offering notable advantages for sustainable livestock production (Aktar et al., 2021). In Türkiye, annual ryegrass is successfully cultivated as a forage crop across all regions (Table 1), significantly contributing to local forage production based on 2023 data (Anonymous, 2023; Ozkan and Demiroglu Topcu, 2024). Adaptation studies identifying suitable varieties have facilitated the successful introduction of this plant throughout the country (Lale and Kokten, 2020; Demiroglu Topcu et al., 2021; Muhit and Kir, 2022; Tassever and Uslu, 2023; Eser et al., 2024; Yuce et al., 2024).

Table 1. Annual ryegrass distribution by region within the forage crops production of Türkiye in 2023

Regions	Annual Ryegrass		Forage Crops		Ratio	
	Area (da)	Yield (ton)	Area (da)	Yield (ton)	Area (%)	Yield (%)
Aegean	202.407	757.760	4.301.214	16.074.848	4.71	4.71
Black Sea	12.258	29.231	2.658.251	5.766.468	0.46	0.51
Central Anatolia	4.827	21.813	3.442.529	13.041.601	0.14	0.17
Eastern Anatolia	532	1.597	11.270.599	14.468.479	0.00	0.01
Marmara	300.955	1.284.148	3.783.919	13.313.311	7.95	9.65
Mediterranean	12.008	43.875	1.267.382	3.501.996	0.95	1.25
Southeastern Anatolia	3.269	16.094	481.562	1.593.750	0.68	1.01
Total	**536.256**	**2.154.518**	**27.205.456**	**67.760.453**	**1.97**	**3.18**

Several studies have highlighted that ryegrass species are generally more sensitive to drought stress than legumes and other plant species (Pornaro et al., 2020; Rahman et al., 2022). Drought-tolerant genotypes demonstrate resilience by maintaining root growth under water-deficit conditions (Mahmood et al., 2022). Key traits, including deeper root systems, improved osmotic potential, and enhanced photosynthetic water-use efficiency, play significant roles in drought tolerance. Grass species with shorter root systems are less adapted to drought (Matthew et al., 2012). Geneticists and plant physiologists are working to improve root architecture and physiological traits to enhance drought resilience (Comas et al., 2013). Therefore, a comprehensive understanding of stress tolerance traits and their integration into whole-plant strategies is essential to improve drought resilience without compromising quality and yield (Lee et al., 2022).

With the global population projected to reach 10 billion by 2050, the demand for versatile forage crops, such as annual ryegrass, is expected to increase significantly. Therefore, identifying annual ryegrass cultivars with strong germination capacity under stress conditions is crucial for supporting sustainable forage production in regions facing climate challenges and water scarcity. This study aims to evaluate the effects of drought stress on the seed germination and seedling growth traits of tetraploid annual ryegrass cultivars.

2. Materials and Methods

The present study was carried out in 2024 at the Seed Laboratory of the Department of Field Crops, Faculty of Agriculture, Ege University, Türkiye. The experimental material consisted of four tetraploid annual ryegrass (*Lolium multiflorum* Lam.) cultivars: Baqueano, Big Boss, Elif, and Medoacus. The experiment was arranged in a randomized plot design with three replications. Seven osmotic potential levels (0, -0.2, -0.4, -0.6, -0.8, -1.0, and -1.2 MPa) were established using polyethylene glycol (PEG-6000). The required amounts of PEG-6000 were dissolved in distilled water to prepare the solutions for the specified osmotic potentials, while distilled water served as the control treatment (Michel and Kaufmann, 1973). Initially, the thousand-seed weights of the tetraploid annual ryegrass cultivars were determined by measuring the weight of four subsamples, each containing 100 seeds, as per ISTA guidelines (ISTA, 2018). Subsequently, the seeds were surface sterilized by immersing them in a 1.0% sodium hypochlorite (NaClO) solution for 5 minutes. Any residual chlorine was removed by thoroughly rinsing the seeds with distilled water (Magar et al., 2019). For the germination tests, a total of 84 glass petri dishes, each 15 cm in diameter, were utilized. Fifty seeds were evenly distributed onto double-layered Whatman No.1 filter papers placed inside each petri dish. Ten milliliters of polyethylene glycol (PEG 6000) solution, prepared at varying concentrations, were added to the petri dishes. To prevent the reduction in oxygen levels caused by the solutions prepared with polyethylene glycol, the filter papers in the petri dishes were replaced every two days during the experiment. The drought tolerance tests were conducted in a growth chamber maintained at a constant temperature of 20±1 °C under dark conditions for a duration of 10 days (ISTA, 2018). Daily observations were performed to evaluate the germination traits, with seeds being classified as "germinated" when the radicle reached a length of 1-2 mm (ISTA, 2018). The number of germinated seeds was recorded and expressed as a percentage. Mean germination time (MGT) was calculated to assess the rate of germination (Ellis and Roberts, 1980). At the end of the 10th day, shoot and root lengths, and seedling fresh weights were measured on ten randomly selected seedlings. Root and shoot lengths were determined using a millimeter ruler, and the shoot-to-root ratio was calculated based on their lengths. Vigor index was calculated by multiplying the sum of the root and shoot length by the germination rate (Hu et al., 2005). Additionally, drought tolerance index was calculated using the respective formula:

Drought Tolerance Index = (Seedling fresh weight at D_x / Seedling fresh weight at D_0) x 100

D_x: Drought levels, D_0: Control

All data were subjected to statistical analysis using analysis of variance (ANOVA) performed in the Statistical Analysis System (SAS Institute, 2012). Differences were considered statistically significant at a probability level of 0.05 or less. Significant differences among treatment means identified through ANOVA were further analyzed using the least significant difference (LSD) test to distinguish them (Johnson and Bhattacharyya, 2019). Furthermore, Pearson's correlation analysis was conducted, as described by Crawford (2006), to evaluate the relationships between the parameters under drought stress conditions.

3. Results and Discussion

In accordance with ISTA (2018) guidelines, the thousand-seed count was performed in four replicates for each tetraploid annual ryegrass cultivars, and the thousand-seed weight was subsequently calculated (Figure 1). Of the four tetraploid annual ryegrass cultivars evaluated, cv. Big Boss exhibited the highest average thousand-seed weight at 4.90 g, while cv. Baqueano showed the lowest at 2.95 g.

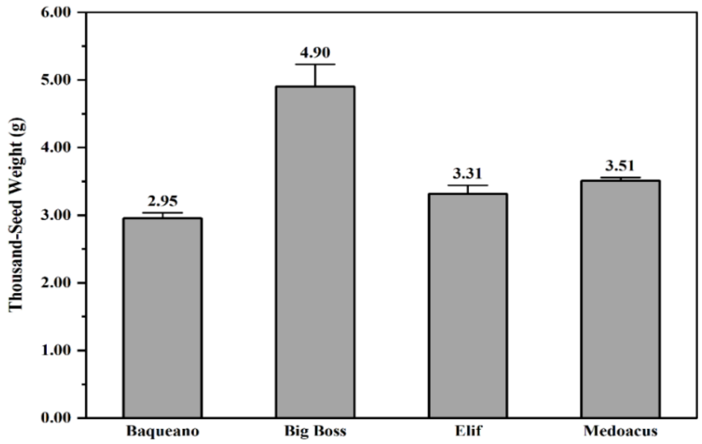

Figure 1. Thousand-seed weight values of examined annual ryegrass cultivars

The statistical analysis results for germination rate indicated significant differences at the 0.05 level among annual ryegrass cultivars, drought levels, and their interaction (Table 2). The highest average germination rate (69.81%) was recorded for seeds of cv. Big Boss, whereas the lowest average value (59.05%) was obtained from seeds of cv. Medoacus. The present study revealed a significant decrease in germination rate values with increasing drought levels. The highest average germination rates were observed in the control, -0.2 MPa, and -0.4 MPa treatments (94.67, 94.00, and 93.83%, respectively), while the lowest was found at the -1.0 MPa drought level (31.83%). No germination was observed at -1.2 MPa drought level. The interaction values are presented in Figure 2. The data revealed that different drought levels had statistically significant effects on the mean germination time of annual ryegrass cultivars (Table 2). In this study, the highest average mean germination times were recorded from the Medoacus and Elif cultivars (3.65 and 3.55 days, respectively). The lowest average mean germination times were observed in the Baqueano and Big Boss cultivars (3.16 and 3.28 days, respectively). A substantial increase in average mean germination time was observed across the different annual ryegrass cultivars as drought levels increased. As expected, the shortest average mean germination time was obtained from the control treatment (2.86 days), while the longest was recorded at -1.0 MPa treatment (5.82 days). Interaction effects are displayed graphically in Figure 2.

Table 2. The effects of drought stress on germination and early growth traits of annual ryegrass cultivars

Treatments	GR (%)	MGT (day)	SL (cm)	RL (cm)	SRR	SFW (mg)	VI	DTI (%)
Cultivars (C)								
Baqueano	67.62[b]	3.16[b]	3.93	5.47[b]	0.46[b]	37.72[a]	872.2[b]	49.60[a]
Big Boss	69.81[a]	3.28[b]	3.81	6.18[a]	0.46[b]	30.10[b]	952.8[a]	44.32[b]
Elif	63.52[c]	3.55[a]	3.86	3.86[c]	0.61[a]	26.82[c]	685.4[d]	42.86[c]
Medoacus	59.05[d]	3.65[a]	4.05	5.30[b]	0.37[c]	29.28[b]	813.5[c]	42.97[c]
Drought Levels (D)								
Control	94.67[a]	2.86[e]	10.15[a]	10.17[a]	1.03[a]	70.19[a]	1924.7[a]	100.00[a]
-0.2 MPa	94.00[a]	2.90[de]	8.80[b]	9.30[b]	1.00[a]	62.79[b]	1699.8[b]	89.16[b]
-0.4 MPa	93.83[a]	3.14[d]	5.28[c]	7.83[c]	0.69[b]	40.77[c]	1236.9[c]	64.65[c]
-0.6 MPa	88.00[b]	4.03[c]	2.88[d]	6.16[d]	0.49[c]	31.44[d]	795.8[d]	44.50[d]
-0.8 MPa	52.67[c]	5.11[b]	0.28[e]	2.27[e]	0.13[d]	9.26[e]	136.1[e]	12.91[e]
-1.0 MPa	31.83[d]	5.82[a]	0.00[e]	0.68[f]	0.00[e]	2.39[f]	23.5[f]	3.35[f]
-1.2 MPa	0.00[e]	0.00[f]	0.00[e]	0.00[g]	0.00[e]	0.00[f]	0.0[f]	0.00[g]
ANOVA								
C	**	**	ns	**	**	**	**	**
D	**	**	**	**	**	**	**	**
C x D	**	**	**	**	**	**	**	**

*p-value<0.05, **p-value<0.01, ANOVA: analysis of variance, ns: not significant

[a,b,c]Means followed by different letters are significantly different (p-value<0.05) according to LSD's test.

GR: germination rate, MGT: mean germination time, SL: shoot length, RL: root length, SRR: shoot-to-root ratio, SFW: seedling fresh weight, VI: vigor index, DTI: drought tolerance index

Germination is generally defined as the process that begins with sowing and culminates in the complete emergence of the coleoptile (El-Kassaby and Edwards, 2001). This period encompasses three distinct phases: seed germination, root growth, and seedling growth. A comprehensive understanding of these interconnected phases is crucial for effectively evaluating drought resistance in crops (Parvez et al., 2003). Key parameters, such as germination rate, shoot and root length, and seedling weight, serve as critical indicators for assessing drought resistance during the germination stage (Almaghrabi, 2012).

Polyethylene glycol molecules influence germination and seedling growth by preventing the entry of water molecules into plant tissues, thereby causing physiological drought (Kalafetoglu Macar et al., 2009). Research indicates that germination rates decrease as drought stress intensifies, with some species showing no germination under -1.2 MPa drought conditions (Rouhi et al., 2011; Borawska-Jarmułowicz et al., 2017). Moderate drought stress is reported to primarily increase the average germination time, whereas severe drought stress significantly reduced germination rates (Demiroglu Topcu and Ozkan, 2016; Bilgili et al., 2019). The decline in germination rates under increasing drought stress has been widely reported by researchers (Demiroglu Topcu and Ozkan, 2016; Hellal et al., 2018; Yilmaz and Kisakurek, 2020; Sari, 2023). Consistent with these findings, present results showed that higher drought levels significantly reduced the germination rate while prolonging the mean germination time in tetraploid annual ryegrass cultivars.

The statistical results related to shoot lenght are arranged in Table 2. The results of the statistical analysis indicated significant differences between drought levels and the cultivar × drought level interaction, whereas cultivar effects were non-significant. Average shoot length values for cultivars ranged between 3.93 and 4.05 cm. The significance of drought levels suggested a decreasing trend in shoot length as drought levels increased. The control treatment showed the longest average shoot length (10.15 cm), while the shortest average value was recorded at -0.8 MPa treatment (0.28 cm). No shoot length was detected at -1.0 MPa treatment. The interaction effects are illustrated in Figure 2.

An analysis of the effects of different drought levels on root length in annual ryegrass cultivars revealed statistically significant differences (Table 2). The highest average root length (6.18 cm) was observed in seeds of cv. Big Boss, while the lowest (3.86 cm) was recorded in seeds of cv. Elif. Root length decreased as drought levels increased. The highest average root length (10.17 cm) was obtained under the control treatment, whereas the lowest average value was observed at -1.0 MPa drought level (0.68 cm). The interaction effects are presented graphically in Figure 2.

The effects of different drought levels on the shoot-to-root ratio (based on length) of annual ryegrass cultivars were statistically significant (Table 2). The highest average shoot-to-root ratio was obtained in seeds of cv. Elif (0.61), while the lowest average values were recorded in seeds of cv. Baqueano and Big Boss (0.46 for both). The significance of drought levels indicated a decreasing trend in the shoot-to-root ratio as drought intensity increased. The highest average shoot-to-root ratios were observed in the control and -0.2 MPa treatments (1.03 and 1.00, respectively), while the lowest was recorded at the -0.8 MPa drought level (0.13). The interaction effects are presented in Figure 2.

Drought stress is a main constraint on forage production worldwide, and a better understanding of germination responses to drought stress may aid breeding programs aimed at developing drought-resistant cultivars (Basu et al., 2010). Drought stress reduced both shoot length (Balkan and Genctan, 2013; Borawska-Jarmułowicz et al., 2017) and root length (Berg and Zeng, 2006; Zhang et al., 2018) in various species and cultivars. Additionally, root growth is restricted under drought stress, while shoot growth declines significantly (Lipiec et al., 2013). Furthermore, drought stress simulated using polyethylene glycol during the germination stage was reported to affect the shoot-to-root ratio (based on length) in various plant species (Hellal et al., 2018; Magar et al., 2019). A similar decrease in shoot-to-root ratio values was observed in the present study. However, despite all cultivars maintaining root length under low moisture conditions (Figure 2), the preserved root lengths were insufficient to counteract the damage caused by water deficiency to overall growth traits (Hussain et al., 2016; Onen et al., 2018). These findings emphasize the adverse effects of drought stress on critical early growth traits, which play a crucial role in cultivation and productivity.

Seedling fresh weight varied depending on both cultivars and drought levels, with these differences also significantly affecting their interaction (Table 2). Among the cultivars, the highest average seedling fresh weight was recorded for cv. Baqueano (37.72 mg), while the lowest average value was observed for cv. Elif (26.82 mg). Similar to shoot and root length, seedling fresh weight decreased as drought levels increased. The highest seedling fresh weight (70.19 mg) was recorded in the control treatment, while the lowest (2.39 mg) was observed at -1.0 MPa treatment. Interaction effects are displayed graphically in Figure 2. Seedling growth provides valuable insights into the responses of crops to drought stress (Khodarahmpour, 2011). It is highly sensitive to drought stress, as limited water availability impairs cell expansion, reduces biomass accumulation, and disrupts the development of critical structures, such as shoots and roots, ultimately affecting plant establishment and productivity (Bhattacharya and Vijaya, 2021). Researchers have reported varying responses in seedling (shoot + root) fresh weight to drought stress across different species and cultivars (Rouhi et al., 2011; Castroluna et al., 2014; Budakli Carpici and Erdel, 2015). In this study, significant reductions in seedling fresh weight were observed across all cultivars as the osmotic potential of the polyethylene glycol solution decreased. Polyethylene glycol-induced drought stress during the germination stage has been shown to significantly affect seed germination and seedling growth traits in various plant species (Gholami et al., 2010; Khayatnezhad et al., 2010; Pei et al., 2010; Demiroglu Topcu and Ozkan, 2016; Bilgili et al., 2019; Sari, 2023). The present findings align with previous studies, which reported that drought stress negatively impacts shoot, root, and seedling weights.

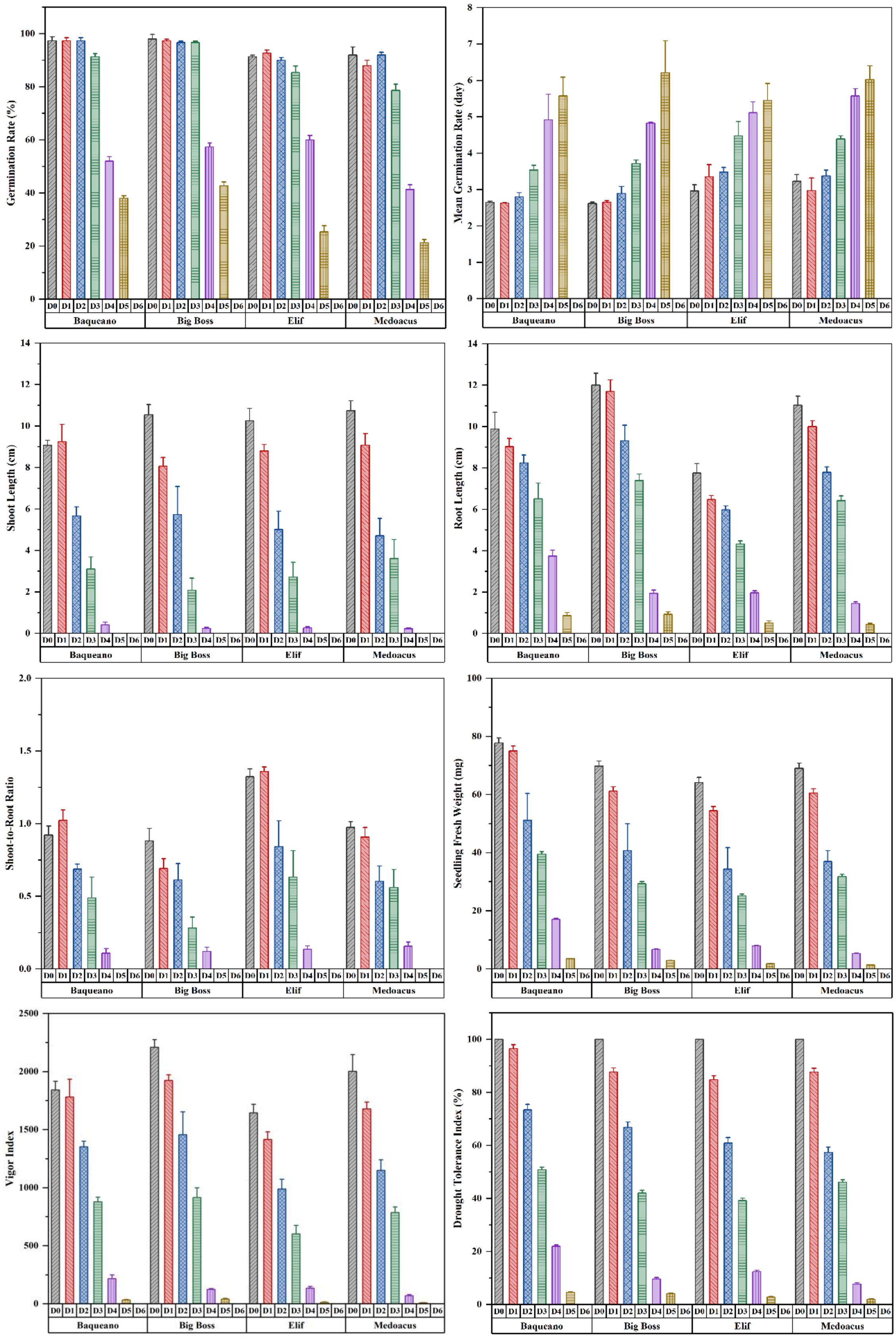

Figure 2. The effects of drought stress on germination and early growth traits of annual ryegrass cultivars
D_0: Control, D_1: -0.2 MPa, D_2: -0.4 MPa, D_3: -0.6 MPa, D_4: -0.8 MPa, D_5: -1.0 MPa, D_6: -1.2 MPa

The effects of different drought levels on the vigor index of annual ryegrass cultivars revealed statistically significant differences (Table 2). In this study, the highest average vigor index was recorded for cv. Big Boss (952.8), while the lowest average vigor index was calculated for cv. Medoacus (813.5). A significant decline in vigor index values was observed as drought levels increased. The highest average vigor index (1924.7) was obtained under the control treatment, whereas the lowest average value (23.5) was observed at -1.0 MPa drought level. Interaction effects are displayed graphically in Figure 2.

The statistical analysis results for the drought tolerance index indicated significant differences at the 0.05 level among annual ryegrass cultivars, drought levels, and the cultivar × drought level interaction (Table 1). The highest drought tolerance index was recorded for seeds of cv. Baqueano (49.60%), while the lowest average values were obtained for seeds of cv. Elif and cv. Medoacus (42.86 and 42.97%, respectively). Although the drought tolerance index tended to decrease after -0.2 MPa drought level, the decline became pronounced at -0.8 MPa level. As expected, the highest average drought tolerance index was recorded in the control treatment (100.00%), while the lowest average value was observed at -1.0 MPa treatment (3.35%). The interaction effects are presented in Figure 2.

The transition from seeds to seedlings, defined as germination, represents a pivotal stage in plant development. Enhanced germination rates under both optimal and challenging environmental conditions support plant growth and increase productivity (Gašparovič et al., 2021). This process depends on the activation of key enzymes and the utilization of stored nutrients essential for embryonic development. However, a decrease in osmotic potential disrupts enzyme functions, leading to a reduction in germination capacity (Billah et al., 2021).

Germination is influenced by climatic conditions and the genetic potential of plants. Therefore, selecting appropriate cultivars is crucial for achieving higher yields (Mondal et al., 2023). Differences among cultivars are attributed to genetic variability and their ability to absorb the moisture necessary for germination (Ibrahimova et al., 2021). Conversely, the water absorption process tends to remain incomplete in seeds sensitive to drought. Adequate water availability is essential for cellular metabolic activities, although the specific water requirements differ across species and cultivars (Larson and Kiemnec, 2005). Starch-derived compounds, including glucose, play a crucial role in germination by acting as osmolytes to sustain cellular turgor and as energy providers (Li et al., 2014). However, under water-deficient conditions, the breakdown of starch is hindered, resulting in suboptimal germination (Kaur et al., 2002).

In the present study, reduced seed germination at higher negative osmotic potentials was associated with lower water imbibition and decreased enzyme activity essential for germination. Significant differences in vigor index and drought tolerance were observed among the studied cultivars, with Baqueano and Big Boss cultivars identified as the most tolerant. An osmotic potential of -0.6 MPa reduced the drought tolerance index of tetraploid annual ryegrass cultivars by up to 44.5%, while almost no drought tolerance (3.35%) was observed at -1.0 MPa. Researchers have indicated that vigor and the drought tolerance index decrease with increasing drought levels (Demiroglu Topcu and Ozkan, 2016; Hellal et al., 2018; Bilgili et al., 2019; Magar et al., 2019). The present study aligns with these findings, confirming that increasing drought levels lead to declines in vigor and drought tolerance index values.

The correlation among the studied traits is presented in Figure 3. The Pearson correlation analysis revealed significant relationships among the evaluated traits, providing insights into their interconnections under drought stress conditions. Germination rate exhibited strong positive correlations with shoot length (r = 0.77), root length (r = 0.89), shoot-to-root ratio (r = 0.81), seedling fresh weight (r = 0.84), vigor index (r = 0.84), and drought tolerance index (r = 0.86). These findings suggest that higher germination rates are associated with improved seedling growth and enhanced drought tolerance. Similarly, shoot and root length were highly correlated with other traits, including shoot-to-root ratio, seedling fresh weight, vigor index, and drought tolerance index. Shoot-to-root ratio also exhibited strong correlations with seedling fresh weight (r = 0.91), vigor index (r = 0.89), and drought tolerance index (r = 0.93), further emphasizing its relevance as an indicator of seedling health and drought adaptation potential. On the other hand, thousand-seed weight exhibited minimal correlations with the evaluated traits, indicating its limited impact on early seedling performance under drought stress. Mean germination time showed weak negative correlations with all other traits, suggesting that prolonged germination adversely affects seedling development and drought tolerance. Consequently, these results underscore the importance of traits such as germination rate, shoot and root length, vigor index, and drought tolerance index as critical indicators for selecting drought-tolerant cultivars.

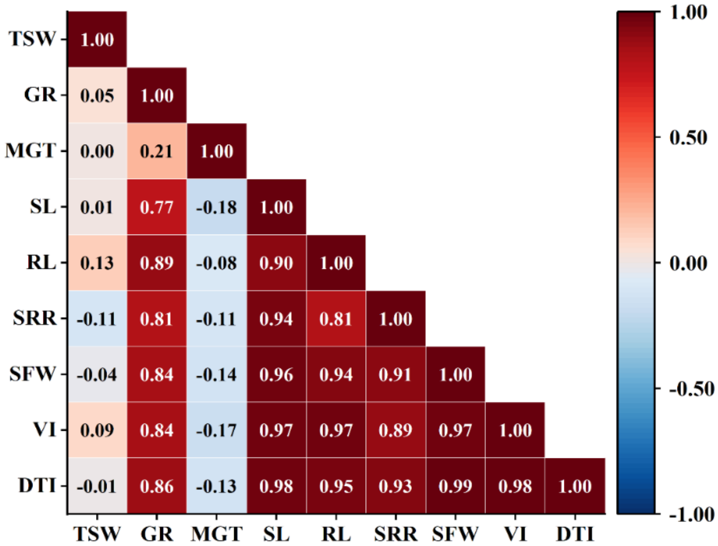

Figure 3. The correlation coefficient of the studied traits

TSW: thousand-seed weight, GR: germination rate, MGT: mean germination time, SL: shoot length, RL: root length, SRR: shoot-to-root ratio, SFW: seedling fresh weight, VI: vigor index, DTI: drought tolerance index

4. Conclusions

Drought stress is one of the most significant challenges to forage production, particularly in regions with limited water availability. Understanding the germination and early growth responses of forage crops under water-deficit conditions is crucial for identifying resilient cultivars. This study demonstrated the significant effects of polyethylene glycol (PEG)-induced drought stress on the germination and seedling growth traits of tetraploid annual ryegrass cultivars. The results indicated that increasing osmotic potential negatively affected critical traits, including germination rate, shoot and root length, seedling fresh weight, vigor index, and drought tolerance index. Among the investigated cultivars, Baqueano and Big Boss cultivars exhibited superior drought tolerance, maintaining higher vigor and drought tolerance indices under water-limited conditions. An osmotic potential of -0.6 MPa reduced the drought tolerance index by up to 44.5%, while no germination was observed at -1.2 MPa. Additionally, strong correlations were identified among germination rate, shoot and root length, vigor index, and drought tolerance index. These findings may provide valuable information for breeding programs focused on developing drought-tolerant annual ryegrass cultivars, thereby supporting sustainable forage crops production in regions prone to water scarcity. Further studies are recommended to investigate the genetic and physiological mechanisms underlying drought tolerance in annual ryegrass.

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.

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