

## Response of Italian Ryegrass (*Lolium multiflorum* L.) Cultivars to Different Drought Levels

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**Abstract:** This study aims to determine the germination and seedling growth responses of Italian ryegrass (*Lolium multiflorum* L.) cultivars under drought stress. The research was conducted in the Laboratory of the Department of Field Crops, Faculty of Agriculture, Siirt University. The plant material used in the study consisted of three Italian ryegrass cultivars Çiğdem, Elif, and Zeybek-19. These cultivars were germinated under different drought levels (0%, 5%, 10%, 15%, and 20%). The laboratory experiment was conducted with a randomized split-plot design, with four replications. In the study, germination percentage (GP), mean germination time (MGT), germination index (GI), germination of uniformity coefficient (CUG), germination energy (GE), seedling fresh weight (SFW), and seedling vigor index (SVI) parameters were examined. The increase in drought levels significantly ( $p<0.01$ ) affected the germination and seedling characteristics of the Italian ryegrass cultivars. The cultivars had a statistically significant effect ( $p<0.01$ ) on the GP, GE, SFW, and SVI. The drought values of the GP, MGT, CUG, GI, GE, SFW, and SVI parameters ranged from 49.8-89.8%, 1.9-3.4 days, 15.0-49.0, 4.8-15.8, 4.4-47.6, 22.3-38.0 mg, and 1.1-3.4, respectively, while the variety averages varied between 70.9-78.7%, 2.1-2.2 days, 38.5-40.4, 12.3-12.6, 28.0-38.4, 28.3-33.5 mg, and 2.1- 2.7, respectively. The effects of drought stress were observed in the germination and seedling growth parameters starting from the 10% drought level. According to the results of the study, the stress tolerance exhibited by the cultivars in response to drought levels also varied. Among the Italian ryegrass cultivars Çiğdem and Elif were more tolerant to drought in terms of germination and seedling growth parameters compared to the other cultivar. In areas experiencing drought, it is important to use drought-tolerance cultivars in Italian ryegrass cultivation, taking into account the genotypic differences.

**Keywords:** *Lolium multiflorum* L., drought stress, germination percentage, seedling fresh weight, seedling vigor index

### 1. Introduction

Italian ryegrass (*Lolium multiflorum* L.) is known for its high forage yield, nutritional value, and richness in mineral content and water-soluble carbohydrates (Humphreys et al., 2010; Açıkbş et al., 2024; Hazarika et al., 2024), and is an important forage crop considered for ruminants in the form of green or dry hay and silage (Goettelmann et al., 2024). In addition, Italian ryegrass is an annual, cool-season grass that is well-suited to temperate and mild climate regions, with the ability to regrow after grazing or cutting (Açıkbş and Özyazıcı, 2023). Furthermore, Italian ryegrass stands out due to its rapid growth, long growing season, and high yield when sufficient nutrients are provided under

suitable environmental conditions (Aganga et al., 2004). Therefore, especially considering the environmental factors associated with global climate change, the use of cultivars with suitable genetic traits is of great importance today.

One of the biggest issues faced in agricultural fields in recent years is drought. In this regard, Kalefetoğlu and Ekmekçioğlu (2005) highlighted that drought stress accounts for the largest share, with 26%, among the stress factors encountered in arable lands worldwide. Additionally, it is predicted that climate change will intensify weather events, resulting in more frequent droughts (Carrasco-Chilón et al., 2024). Drought is a significant environmental stress, especially caused by low

rainfall due to climate change (Seleiman et al., 2021). Drought stress directly affects plant growth, development, and yield potential, leading to a decrease in global agricultural production (An and Liang, 2012; Anjum et al., 2017; Çiğ et al., 2021; Yasmeen et al., 2022; Asma et al., 2023).

Drought leads to various negative effects by disrupting plant processes, such as a reduction in carbon assimilation rates, loss of turgor, increased oxidative damage, and changes in leaf gas exchange (Chowdhury et al., 2016; Hussain et al., 2018). In addition, drought affects various morpho-physiological traits of plants during all stages of growth (Wei et al., 2018), with germination and seedling stages, which are particularly sensitive to drought stress, directly influencing the plant's later growth and yield stages (Yousefi et al., 2020). In semi-arid regions where drought issues are prevalent, it is important to develop plant species that can withstand water stress at different growth stages or to use drought-tolerant varieties (Tiryaki, 2016). This study aims to explore the germination and seedling growth responses of Italian ryegrass (*L. multiflorum*) cultivars under different drought levels.

## 2. Materials and Methods

### 2.1. Material

The research was conducted in the Laboratory of the Department of Field Crops, Faculty of Agriculture, Siirt University. In the study, the plant materials used were Çiğdem, Elif, and Zeybek-19 Italian ryegrass (*L. multiflorum*) cultivars. Drought stress conditions were provided using polyethylene glycol (PEG-6000).

### 2.2. Experimental design

The topic of the study involved applying five different drought levels (0%, 5%, 10%, 15%, and 20%) to three Italian ryegrass cultivars. The laboratory experiment was designed according to a split-plot design in randomized complete blocks with four replications. When selecting the concentrations, the osmotic resistance of the PEG-6000 solution, which ranged from -0.3 to -0.80 MPa, was considered as the drought stress range

(Muscolo et al., 2014). The drought stress levels were determined by preparing solutions with concentrations of 0%, 5%, 10%, 15%, and 20% based on weight/volume.

In each Petri dish (90 mm x 15 mm), 25 seeds of uniform size were placed between two layers of filter paper for sowing. Initially, 5 ml of solution (PEG-6000 solution) was applied to each Petri dish, and in the following days, appropriate drought solutions were added according to the dryness condition of the Petri dishes. The Petri dishes were left to germinate in a dark environment at a temperature of 25±1 °C for 10 days. The number of germinated seeds was recorded daily throughout the 10 days. Germination was determined based on the criteria reported by Scott et al. (1984) and Soleymani and Shahrajabian (2018), where plants with a rootlet of at least 2 mm were considered germinated.

### 2.3. Measurement method for germination and seedling growth parameters

Measurements were made on 10 randomly selected plants from each Petri dish, and in Petri dishes where there was insufficient germination due to drought stress, measurements were made only on the germinated plants. The study examined germination characteristics, including germination percentage (GP), mean germination time (MGT), coefficient of uniformity of germination (CUG), germination energy (GE), and germination index (GI), as well as seedling characteristics such as seedling fresh weight (SFW) and seedling vigor index (SVI). The formulas and references for the germination and seedling growth parameters examined in the study are provided in Table 1. The SFW were determined by weighing the seedlings, which were cleaned of surface water and weighed on a precision balance.

### 2.4. Statistical analysis

The obtained data were subjected to analysis of variance according to the randomized split-plot design, and the differences between the means were checked by Tukey's multiple comparison test (Açıkgöz and Açıkgöz, 2001).

**Table 1.** Formulas and references related to some of the parameters examined

Examined parameters	Equation	Equality explanation	References
Germination percentage	$(NGS/TS) \times 100$	NGS is the number of normally germinated seeds, TS is the total number of seeds used	Scott et al. (1984)
Mean germination time	$\Sigma(N_i T_i / N_i)$	$N_i$ is the number of seeds germinated on day $T_i$ , $T_i$ is the number of days counted from the beginning of germination	Ellis and Roberts (1981)

**Table 1.** (Continued)

Examined parameters	Equation	Equality explanation	References
Coefficient of uniformity of germination	$\Sigma n/\Sigma[(MGT-t)^2n]$	$t$ is the time in days, starting from day 0, the day of sowing, $n$ is the number of seeds completing germination on day $t$	Bewely and Black (1994)
Germination energy	$(T_1/N) \times 100$	$T_1$ is the number of seeds germinated on the first day, $N$ is the total number of seeds	Li et al. (2020)
Germination index	$\Sigma(G_i/T_i)$	$G_i$ is the germination percentage on the $i$ th day $T_i$ is the days of the germination test	Wang et al. (2004)
Seedling vigor index	GPxSFW	GP: Germination percentage, SFW: Seedling fresh weight	Kalsa and Abebie (2012)

### 3. Results

#### 3.1. Analysis of variance experimental data

The results of the variance analysis for the germination and seedling parameters of the Italian ryegrass varieties at different drought levels are presented in Table 2.

**Table 2.** Analysis of variance for treatments on investigated characteristics

Traits/ Factors	TUKEY value/F probability		
	Cultivars (C)	Drought levels (DL)	CxDL
GP	2.51**	3.81**	8.39**
MGT	0.21 <sup>ns</sup>	0.32**	0.70 <sup>ns</sup>
CUG	4.64 <sup>ns</sup>	7.05**	15.54 <sup>ns</sup>
GE	6.29**	9.54**	21.09 <sup>ns</sup>
GI	1.03 <sup>ns</sup>	1.56**	3.45 <sup>ns</sup>
SFW	2.64**	4.00**	8.84 <sup>ns</sup>
SVI	0.22**	0.35**	0.78 <sup>ns</sup>

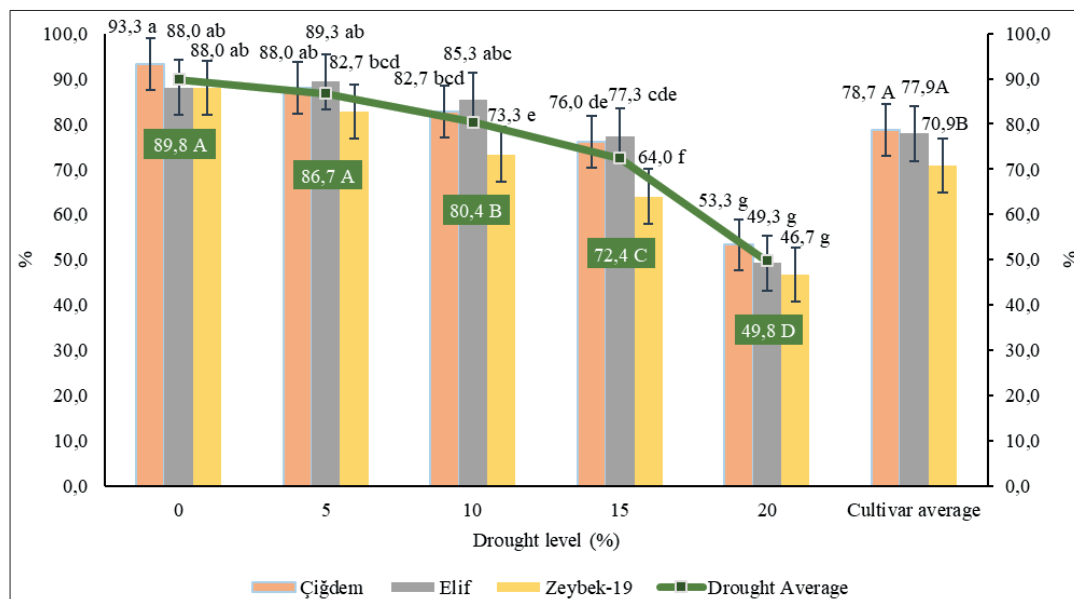
\*\* : Significant difference at the  $p < 0.01$  level, ns: No significant difference

Upon examining Table 2, it was found that the cultivars had a statistically significant effect ( $p < 0.01$ ) on the GP, GE, SFW, and SVI. The effects of the applied drought levels, on all other examined parameters were statistically highly significant ( $p < 0.01$ ). In the study, the drought x cultivar interaction for GP was found to be statistically significant ( $p < 0.01$ ) (Table 2).

#### 3.2. The effect of cultivars and drought levels on germination characteristics

##### 3.2.1. Germination percentage

The data on the GP of Italian ryegrass cultivars at different drought levels are presented in Figure 1. As a result of the study, increasing drought levels reduced the GP, but the difference between the 0% and 5% drought levels was found to be statistically insignificant. Significant decreases occurred after the 10% drought level, with the highest GP being 89.8% and 86.7% at the control and 5% drought

**Figure 1.** Germination percentage of Italian ryegrass cultivars in the different drought levels

levels, respectively. The lowest value was found at the highest drought level (20%), at 49.8% (Figure 1).

When examining the average results of the Italian ryegrass cultivars in terms of GP, the highest GP were found to be 78.7% for the Çiğdem variety and 77.9% for the Elif cultivar, based on the drought levels. The lowest GP, with an average of 70.9%, was observed in the Zeybek-19 cultivar (Figure 1).

In the study, the cultivar x drought interaction was found to be significant in terms of GP. The significance of the interaction is believed to be due to the fact that, despite an increase in drought stress in some cultivars, the GP increased, and values at

higher drought levels were higher than those of different cultivars at lower drought levels (Figure 1).

### 3.2.2. Mean germination time

In the study, the fastest germinations occurred at the 10% drought level in an average of 1.7 days, while this value increased to 3.4 days at the 20% drought level due to increasing drought stress, leading to delayed germination. When examining the effects of the cultivars on the MGT, it was determined that the cultivars showed a variation between 2.1 to 2.2 days on average (Figure 2). In terms of MGT, the effect of drought levels on the cultivars did not result in a statistically significant difference.

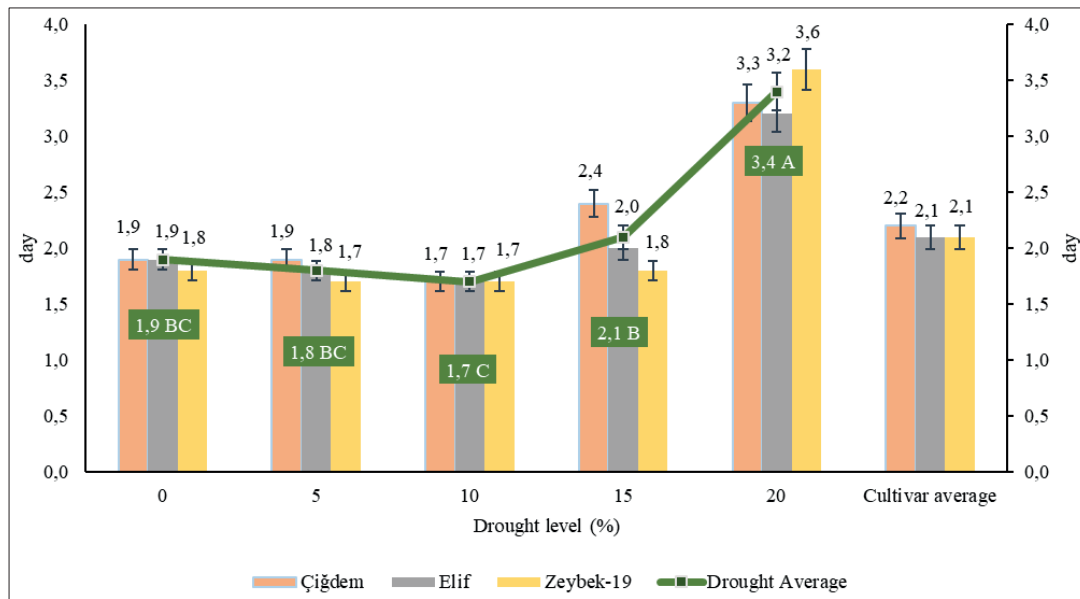


Figure 2. Mean germination time of Italian ryegrass cultivars in the different drought levels

### 3.2.3. Coefficient of uniformity of germination and germination index

Drought stress significantly reduced the CUG and GI with significant decreases starting from the 15% drought level. The highest values for the CUG and GI were observed in the control group (47.9 and 15.4, respectively), the 5% drought level (49.0 and 15.8, respectively), and the 10% drought level (48.0 and 14.8, respectively). The lowest values were found at the 20% drought level (15.0 and 4.8, respectively). In the study, where the difference between cultivars was insignificant, the CUG and GI values varied between 38.5-40.4 and 12.3-12.6, respectively, depending on the varieties (Figure 3 and 4).

### 3.2.4. Germination energy

When examining the average results of the Italian ryegrass cultivars in terms of GE, the highest GE, with an average of 38.4, was determined in the Zeybek-19 cultivar. There was no statistically significant difference between the Zeybek-19 cultivar, with the highest GE value, and the Çiğdem cultivar. The lowest GE was found in the Elif cultivar, with a value of 28.0. As the average of the cultivars in terms of GE, significant differences were observed starting from the 10% drought level. The highest GE values were detected in the control (47.1), 5% (47.6), and 10% (43.6) drought levels. A decrease in GE values was observed with increasing drought levels, with the lowest GE result recorded at the 20% drought level (4.4) (Figure 5).

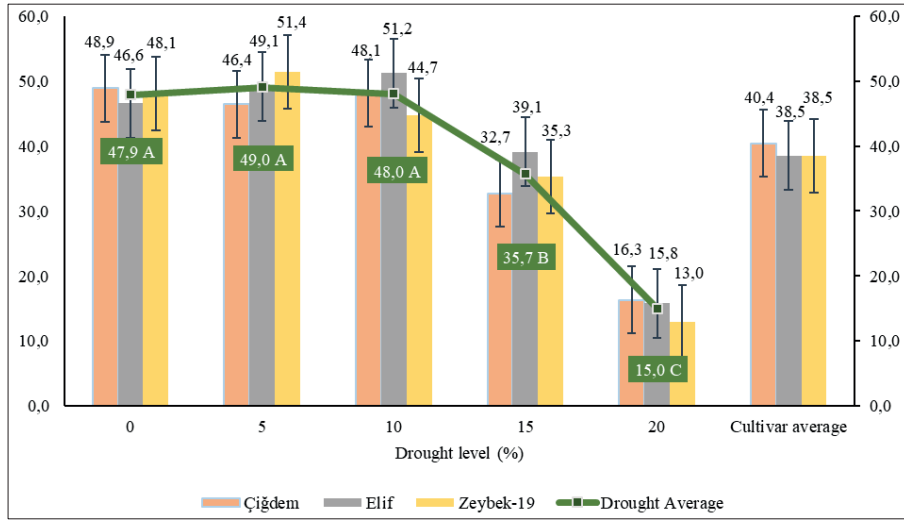


Figure 3. Coefficient of uniformity of germination of Italian ryegrass cultivars in the different drought levels

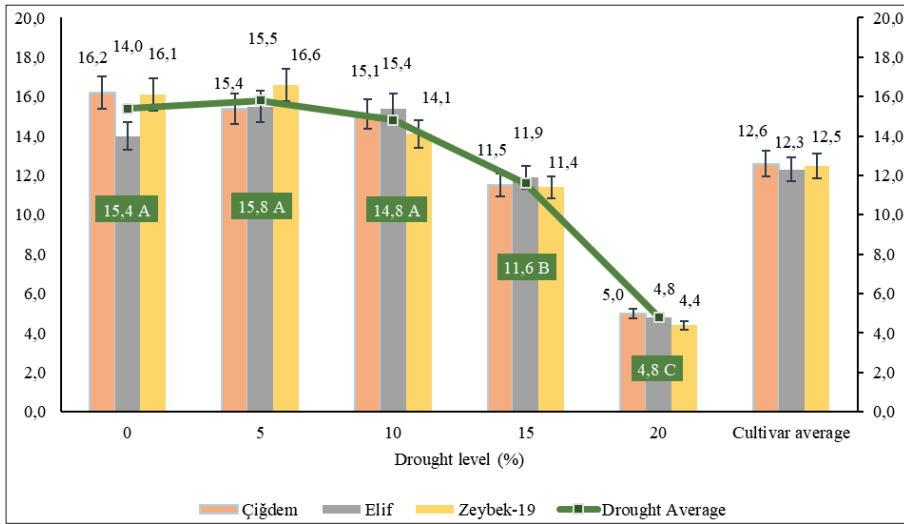


Figure 4. Germination index of Italian ryegrass cultivars in the different drought levels

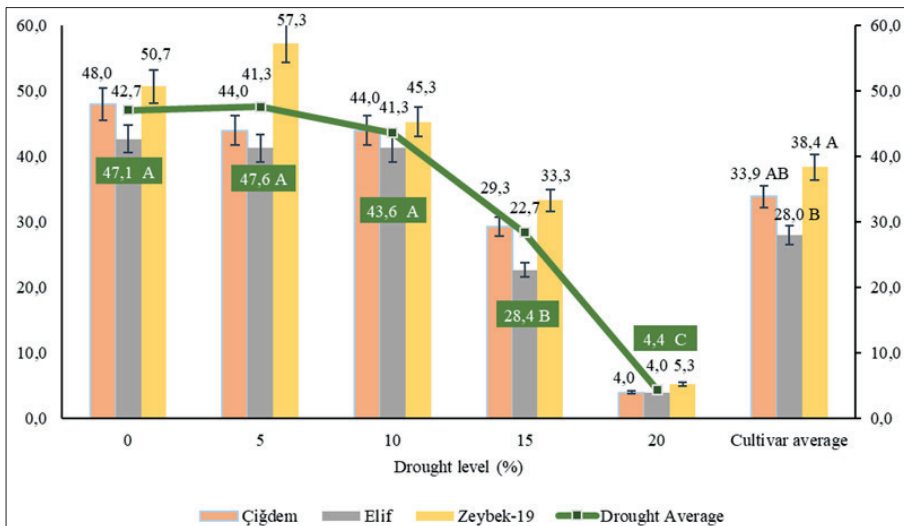


Figure 5. Germination energy of Italian ryegrass cultivars in the different drought levels

### 3.3. The effect of cultivars and drought levels on some seedling development parameters

#### 3.3.1. Seedling fresh weight

When examining the SFW, one of the seedling development parameters, significant effects from drought stress were observed starting from the 10% drought level. As the drought levels increased, reductions in SFW were noted. The highest value (38.0 mg) were obtained in the control group (0% drought), with no statistical difference observed at the 5% drought level. The lowest values were found at the highest drought level, 20%, with a value of 22.3 mg. When the average values of the cultivars were examined, the cultivars with the highest SFW, according to the average results of the drought

levels, were Çiğdem (33.5 mg) and Elif (31.7 mg). The variety with the lowest SFW was Zeybek-19 (28.3 mg) (Figure 6).

#### 3.3.2. Seedling vigor index

When examining another seedling parameter, the SVI, it was observed that increasing drought stress negatively affected the SVI starting from the 10% drought level. The highest results were found in the control group and the 5% drought level, while the lowest results were observed at the highest drought level, 20%. When examining the average values of the cultivars for the SVI, the highest values were recorded for Çiğdem (2.7) and Elif (2.6). The cultivar with the lowest SVI was Zeybek-19 (2.1) (Figure 7).

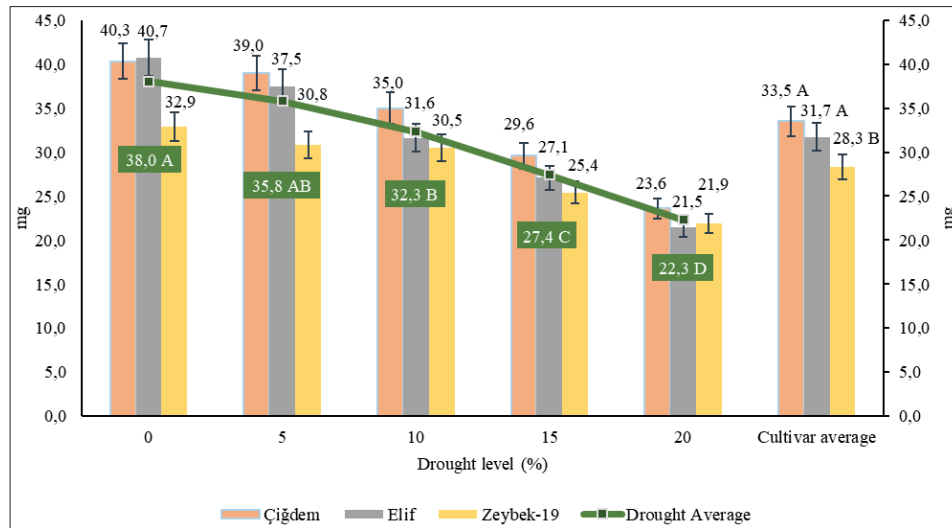


Figure 6. Seedling fresh weight of Italian ryegrass cultivars in the different drought levels

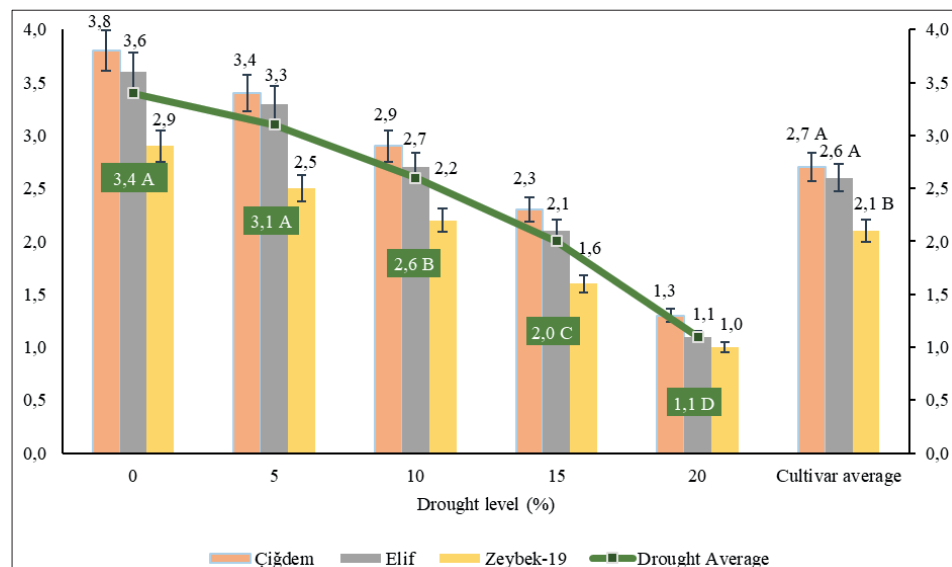


Figure 7. Seedling vigor index of Italian ryegrass cultivars in the different drought levels

#### 4. Discussion and Conclusion

Although it varies among plant species, germination, early seedling development, and flowering stages are the most sensitive periods to drought (Windauer et al., 2007; Ahmadi et al., 2009; Çifçi and Açıkbaş, 2023). During the developmental stages of plants, leaf development, enzyme activity, and ion balance are adversely affected by drought stress, impacting physiological, biochemical, and molecular processes (Anjum et al., 2017; Todaka et al., 2017; Wang et al., 2018; Çiğ et al., 2022). When seeds do not receive sufficient water, both the hydrolysis process of carbohydrates and the activity of amylase enzymes are damaged, leading to delays in the germination process or its complete cessation (Zeid and Shedeed, 2006).

In a study investigating the effects of different drought stress levels created with PEG 6000 on the germination characteristics of four grass forage species (*Festuca ovina*, *Festuca arundinacea*, *Agropyron cristatum*, and *Bromus inermis*), it was reported that the GP decreased in all species studied as drought levels increased (Rouhi et al., 2011). Similar results have been reported in studies where artificial drought (PEG-6000) was applied to different plant species, showing a decrease in GP as drought stress increased, similar to the findings of our study (Van den Berg and Zeng, 2006; Farooq et al., 2009; Borawska-Jarmułowicz et al., 2017; Yılmaz et al., 2022; Turan and Samur, 2024). It was observed that the response of varieties to drought stress in terms of GP varied. Indeed, it has been reported that *Lolium perenne* varieties also exhibit different responses to drought stress (Yılmaz and Kısakürek, 2020). Borawska-Jarmułowicz et al. (2017) reported that as the drought level increased, the germination time extended for *Lolium perenne* L. and meadow brome grass varieties. The findings of this study are supported by similar results obtained by Khodarahmpour (2011) in maize, Aslan and Atış (2018) in grasspea, and Çifçi and Açıkbaş (2023) in common vetch. Among the germination parameters examined, the CUG, GI, and GE were affected starting from the 15% drought level, and negative effects were observed at higher levels. Similar results have been reported in various studies (Gürbüz et al., 2009; Aydın et al., 2015; Aslan and Atış, 2018). It has been reported that SFW decrease with the increase in drought stress in various studies, including those on forage pea (Uslu et al., 2021), wheat (Khan et al., 2013; Khan et al., 2019), sorghum (Bibi et al., 2012), perennial ryegrass (Yılmaz and Kısakürek, 2020), and alfalfa (Castroluna et al., 2014; Molor et al., 2016).

Abiotic stresses occurring during the germination phase, which is one of the most critical stages of plant development, significantly affect the growth, productivity, and quality of plants in later stages. In terms of the characteristics examined in the study, it was observed that the cultivars were affected differently by drought levels. Additionally, varieties that exhibited drought tolerance during the germination stage did not demonstrate a similar tolerance in more advanced developmental stages. In studies conducted with different plant species and cultivars (Rouhi et al., 2011; Ahmad et al., 2014), it has been reported that different species and cultivars within the same species show different responses to drought stress in terms of germination and seedling development characteristics.

Significant differences were found among the varieties in terms of GP, GE, SFW, and SVI, and it is believed that these differences arise from the genotypic structure of the varieties. Similar results have been obtained in drought studies conducted with different plant species using the same parameters (Okçu et al., 2005; Türkan et al., 2005; Arslan et al., 2018; Uslu et al., 2021). It has been emphasized that to combat drought stress, resistant/tolerant species and varieties should be developed with the help of traditional breeding methods, biotechnological applications, and molecular-assisted selection technologies (Samancıoğlu and Yıldırım, 2015).

The effects of drought stress were observed in germination and seedling development parameters starting from the 10% drought level. According to the research results, the stress tolerance of the cultivars varied in response to the drought levels. Among the Italian ryegrass cultivars, Çiğdem and Elif varieties were more tolerant to drought in terms of germination and seedling development parameters compared to the other cultivar. In areas experiencing drought, it is recommended to use drought-resistant varieties, considering the genotypic differences, in Italian ryegrass cultivation.

#### Ethical Statement

The author declares that ethical approval is not required for this research.

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#### Declaration of Conflicts of Interest

No conflict of interest has been declared by the author.

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