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Tolerance of forage pea cultivars to salinity and drought stress during germination and seedling growth

Gülçin Küçüközcü[1] **Süleyman Avcı[1,*]**

[1]Eskişehir Osmangazi University, Faculty of Agriculture, Department of Field Crops, Eskişehir, Turkey

*Corresponding Author: savci@ogu.edu.tr

Abstract

The germination and seedling characteristics of six forage pea cultivars were investigated under different levels of salinity (0, 5, 10, 15, and 20 dS/m) and drought (0, -2, and -4 bar) stresses. All characteristics of germination and seedling growth varied by cultivar, salinity and drought levels, and their interactions. With high seedling lengths overall, Özkaynak, Ulubatlı, and Töre cultivars demonstrated low reduction rates in seedling length when salinity reached 15 dS/m, whereas the Taşkent cultivar's reduction rate increased considerably at that level of salinity, as did Ürünlü and Gölyazı cultivars. Meanwhile, the Gölyazı cultivar had high fresh and dry weights, despite high reduction rates in fresh weight, similar to the Taşkent cultivar. Though the Ulubatlı cultivar had the shortest seedlings, they exhibited a low reduction rate in seedling length at -2 bar of drought stress, as did Töre and Özkaynak cultivars. At that level of drought stress, those cultivars also indicated low reduction rates in fresh weight. Altogether, the Töre cultivar best tolerated salinity and drought conditions, the Özkaynak cultivar showed promise as well, whereas Taşkent and Gölyazı cultivars were the most sensitive to the conditions.

Keywords: Forage legume, Seedling length, Reduction rate, PEG, NaCl

Introduction

Worldwide, 20% of agricultural lands experience salt stress, and that rate is expected to rise to 50% by 2050 (Kang et al., 2010; Tiryaki, 2018). Salinity in the soil mostly occurs in arid and semi-arid regions with low rates of precipitation and high temperatures, where it causes severe losses in yield (Munns and Termaat, 1986; Umezawa et al., 2001). In studies on salinity, germination and seedling development are typically examined in determining how crop genotypes respond to salt (Ghoulam and Fares, 2001; Kara and Uysal, 2010). The top reason why seeds in overly salty soils face adversity during germination is the stunted intake of water through the seed coat (Coons et al., 1990; Mansour, 1994). Beyond that, deterioration in the ion balance in plants grown in saline soils damages their physiological functions, including their capacity for photosynthesis and respiration (Levitt, 1980; Aydınşakir et al., 2012).

Aside from salinity, drought stress, observed in 26% of agricultural lands worldwide, causes a range of physiological, biochemical, and molecular events in plants (Blum and Jordan, 1985). During drought, plants reduce their cell growth as a means to protect against water deficiency (Taiz and Zeiger, 2015), after which turgor pressure drops, and the water balance between plant tissues becomes disturbed (Levitt, 1980). On top of that, drought not only causes damage to pigments for photosynthesis and decreases chlorophyll content (Saeidi and Abdoli, 2015) but also causes the failure of major organs and root elongation by increasing the amount of absicic acid, which decreases the amount of cytokinin and gibberellic acid (Özel et al., 2016).

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ORCID: Gülçin Küçüközcü 0000-0002-5630-6397 Süleyman Avcı 0000-0002-4653-5567
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In Turkey, barren lands represent 5.48% of all cultivated lands, and 74% of those barren lands are salty; 25.5% have saline–alkaline soils, while 0.5% have alkaline soils (Karaoğlu and Yalçın, 2018). Although the average annual rainfall in Turkey is approximately 640 mm, many regions experience water shortage and drought due to the irregular distribution of precipitation. It has been hypothesized that Turkey ranks among countries at risk to the possible effects of global warming, and in the future, Turkey's Mediterranean and Inner Anatolian regions will be especially more affected by climate change (Kapluhan, 2013). Considering the quality roughage deficit in Turkey, it is critical to identify forage crops resistant to salt and drought stress with high yield potential that can guarantee their use as fodder for livestock in the future.

Among likely candidates, forage peas [(*Pisum sativum* var. *arvense* (L.) Poiret)] produced for fresh forage, hay, and silage have not only shown high yield potential but also been adapted to nearly all of Turkey's regions, regardless of their different climatic and soil conditions (Uzun et al., 2012). Rich in protein, the hay and seed of forage peas are both nutritious and attractive food sources for livestock (Açıkgöz et al., 1985). They also leave clean stubble as well as significant nitrogen and organic matter in the soil for the benefit of subsequent crops (Parr et al., 2011).

Because the efficiency of cultivating forage peas depends on their ability to cope with abiotic stresses, determining the responses of different forage pea varieties against stress factors is critical. Recognizing that need, Avcı et al. (2018) and Demirkol et al. (2019) have investigated the effects of salt stress on the germination and early seedling development of forage peas in Turkey. In other work, Okçu et al. (2005), Petrović et al. (2016), and Pereira et al. (2020) have examined the responses of different pea genotypes to salt and drought stress. In those studies, the responses of pea genotypes to salt and drought stress differed, however. In response to those findings, the study reported here involved examining how salt and drought stress affect the germination and seedling growth of six different forage pea cultivars in Turkey.

Materials and Methods

Three purple-flowered (Töre, Taşkent, and Özkaynak) and three white-flowered (Ulubatlı, Ürünlü, and Gölyazı) forage pea (*P. sativum* var. *arvense* L. Poir.) cultivars were used as seed materials.

A controlled experiment in incubators was performed in a two-factor arrangement with a completely randomized design involving four replications. The first factor was the forage pea cultivars under both stresses, while the second was salt (0, 5, 10, 15, and 20 dS/m) or drought (0, -2, and -4 bar) stress. Salinity was adjusted with a WTW 3.15i EC meter using sodium chloride (NaCl), whereas drought stress was achieved using polyethylene glycol (PEG 6000 mol.w.), as described by Michel and Kaufmann (1973).

Germination was performed in four replications of 50 seeds each on three filter papers 20 × 20 cm in size (ISTA, 2018). Once 7 mL of pure water was added to each filter paper, the papers were placed in sealed plastic bags in order to prevent evaporation. The papers were checked every 2days, and pure water was added as needed. The seeds were counted every day, and ones with a root length of 2 mm were considered to have germinated.

Germination percentage, mean germination time, seedling length, seedling fresh and dry weights, and reduction rate were evaluated to determine the tolerance of the cultivars to salinity and drought. Mean germination time was calculated with the formula of Ellis and Roberts (1980), while the reduction rate was calculated as follows: (Seedling characteristics of control plants – Seedling characteristics of stress plants) / (Seedling characteristics of control plants) ´ 100.

All data were subjected to variance analysis using MSTAT. Arcsin √x transformation was applied to percentage values (Sokal and Rohlf, 1981), and Duncan's multiple comparison test was performed to determine the differences, if any, between mean values.

Results and Discussion

In terms of salt stress, the effects of cultivars, salinity, and the interaction of cultivar and salinity on characteristics of germination and seedling growth were significant at the 1% level (Table 1). As shown in Table 2, the Töre cultivar in the control condition and in 5 dS/m of salinity, along with the Gölyazı cultivar in the control condition and in 5 and 10 dS/m of salinity, had higher germination percentages than the other cultivars. The lowest germination percentage, was recorded for the Ulubatlı cultivar, decreased rapidly once salinity reached 5 dS/m. In general, the increase in salinity began adversely affecting germination at levels of 10 dS/m and higher (Table 1). Similar to those findings, Demirkol et al. (2019) observed a significant decrease in germination rate parallel to increased salinity after 90 mM. Contrary to those findings, Okçu et al. (2005) and Avcı et al. (2018) reported that germination percentage did not vary by level of salinity.

Mean germination time was prolonged in all cultivars as salinity level increased (Table 2). Whereas Töre, Özkaynak, and Ürünlü cultivars germinated the fastest, Ulubatlı and Gölyazı cultivars were the slowest (Table 1). Such findings confirm the results of Tsegay and Andargie (2018) and Demirkol et al. (2019), who found that mean germination time increased in parallel to salt concentration. Similar findings have also been reported for lentils (Karaman and Kaya, 2017), common vetch (Ertekin et al., 2017), and Hungarian vetch (Önal Aşçı and Üney, 2016).

As salinity rose, the length of seedlings decreased, except in Özkaynak and Ulubatlı cultivars (Table 2). In those cultivars, the effect of salinity at 5 dS/m on seedling length was even positive. When the reduction rate in seedling length compared to the control at 15 dS/m was evaluated, Özkaynak, Ulubatlı, and Töre cultivars demonstrated the lowest values, in that order (Figure 1). However, a dose of 20 dS/m negatively affected nearly all varieties, for the average reduction rate of approximately 70%. Avcı et al. (2018) and Demirkol et al. (2019) also found that salinity negatively affected seedling growth, as well as that its effect on the roots of pea genotypes commenced at lower doses than on the shoots.

The highest fresh and dry weights of all seedlings were obtained in the control condition for the Gölyazı cultivar, whereas the lowest were obtained in the 20 dS/m treatment of the Taşkent cultivar (Table 2). As salinity increased, fresh and dry weights decreased in all cultivars except for Özkaynak, whose fresh and dry weights were positive affected by 5 dS/m of salinity. The lowest reduction rate in fresh weight at 15 dS/m of salinity was recorded in that cultivar as well (Figure 2). Although all reduction rates in the fresh weights of the seedlings at 20 dS/m were similar, the Taşkent cultivar was the most affected. Supporting those findings, Avcı et al. (2018), Tsegay and Andargie (2018), and Demirkol et al. (2019) have all reported that the fresh and dry weights of seedlings decreased in pea genotypes due to increased salinity.

Table 1. Analysis of variance and differences between mean values of germination and seedling growth characters of forage pea cultivars grown under different salinity stresses.

Factors	Germination (%)	Mean germination time (day)	Seedling length (cm)	Fresh weight (mg/ seedling)	Dry weight (mg/ seedling)
Cultivars					
Töre	99.60[a†]	2.85[c]	9.83[a]	138.89[bc]	13.31[cd]
Taşkent	94.80[b]	2.99[b]	9.30[a]	132.06[c]	13.03[d]
Özkaynak	95.10[b]	2.98[bc]	9.93[a]	142.37[b]	13.93[cd]
Ulubatlı	94.50[b]	3.15[a]	9.48[a]	154.14[a]	15.39[b]
Ürünlü	95.20[b]	2.96[bc]	8.45[b]	137.30[bc]	14.08[c]
Gölyazı	98.90[a]	3.18[a]	8.18[b]	161.16[a]	16.70[a]
Salinity (dS/m)					
0 (Control)	97.91[a]	2.62[d]	13.38[a]	249.21[a]	22.04[a]
5	96.50[a]	2.95[c]	12.83[a]	179.18[b]	17.27[b]
10	97.58[a]	3.06[bc]	9.52[b]	139.06[c]	13.93[c]
15	95.33[b]	3.13[b]	6.23[c]	86.77[d]	10.20[d]
20	94.41[b]	3.35[a]	4.02[d]	67.37[e]	8.58[e]
Analysis of variance					
Cultivars (A)	*	*	*	*	*
Salinity (B)	*	*	*	*	*
A x B	*	*	*	*	*

*: significant level of 1%. †: letters show different groups at 5% level.

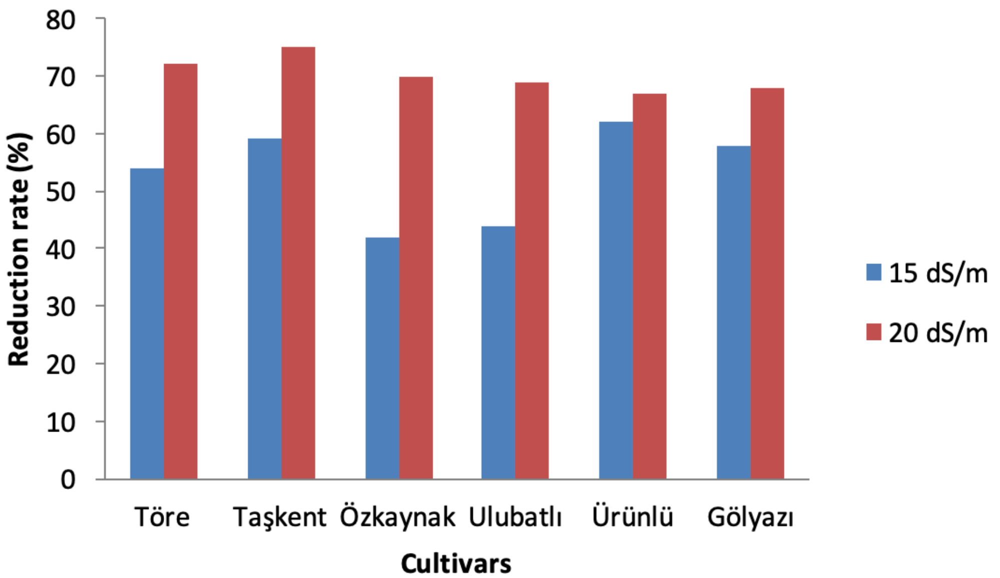

Figure 1.Reduction rates in seedling lengths of forage pea cultivars at 15 and 20 dS/m of salinity stresses

Table 2. The effect of cultivars x salinity interaction on germination and seedling growth characters of forage pea cultivars.

Cultivars	Salinity (dS/m)	Germination (%)	Mean germination time (day)	Seedling length (cm)	Fresh weight (mg/seedling)	Dry weight (mg/seedling)
Töre	Control	100.0	2.38	14.55	229.75	20.25
	5	100.0	2.66	14.16	184.15	16.10
	10	99.0	2.93	9.66	145.02	14.22
	15	100.0	3.12	6.77	76.77	8.37
	20	99.0	3.16	4.01	58.75	7.62
Taşkent	Control	95.0	2.71	14.20	242.00	21.00
	5	90.5	2.73	14.12	171.45	15.92
	10	99.0	3.08	8.68	117.55	12.42
	15	94.5	3.00	5.89	73.80	8.80
	20	95.0	3.46	3.61	55.47	7.02
Özkaynak	Control	96.5	2.36	12.89	206.25	18.50
	5	95.0	2.85	16.04	209.28	18.92
	10	95.5	2.98	9.45	148.85	14.82
	15	95.0	3.16	7.28	89.22	10.27
	20	93.5	3.56	3.98	58.27	7.15
Ulubatlı	Control	99.5	3.04	11.81	256.75	23.50
	5	99.0	3.36	14.20	188.38	18.37
	10	95.5	3.09	10.95	152.75	14.55
	15	89.5	2.97	6.68	96.90	10.97
	20	89.0	3.32	3.76	75.90	9.55
Ürünlü	Control	96.5	2.71	13.34	263.50	22.00
	5	94.5	2.89	9.42	133.15	14.67
	10	96.5	3.00	9.92	135.75	13.32
	15	96.0	3.09	5.17	80.70	11.12
	20	92.5	3.14	4.40	73.37	9.30
Gölyazı	Control	100.0	2.52	13.46	297.00	27.00
	5	100.0	3.20	9.06	188.65	19.67
	10	100.0	3.27	8.45	134.42	14.25
	15	97.0	3.45	5.60	103.25	11.70
	20	97.5	3.48	4.35	82.50	10.87
$LSD_{\%5}$		6.39	0.46	1.63	18.07	1.76

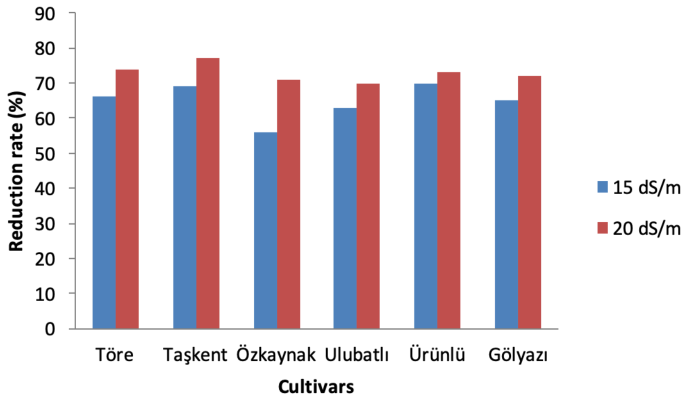

Figure 2. Reduction rates in seedling fresh weights of forage pea cultivars at 15 and 20 dS/m of salinity stresses

As in the salt stress study, the effects of cultivar, drought, and their interaction on characteristics of germination and seedling growth were significant in the drought study (Table 3). The highest and lowest germination percentages were achieved by the Töre and Gölyazı cultivars, respectively, although the germination percentages of the cultivars were not negatively affected in -2 bar of drought stress except in the Ulubatlı and Gölyazı cultivars (Table 4). Those two cultivars also showed the greatest decrease in germination percentage at -4 bar of drought stress. Drought stress caused by PEG negatively affected germination on pea genotypes in the studies of Okçu et al. (2005) and Pereira et al. (2020). Similar results have also been obtained for lentil genotypes (Muscolo et al. 2013), grass pea varieties (Aslan and Atış, 2018), and some alfalfa genotypes (Özkurt et al., 2018).

Table 3. Analysis of variance and differences between mean values of germination and seedling growth characters of forage pea cultivars grown under different drought stresses.

Factors	Germination (%)	Mean germination time (day)	Seedling length (cm)	Fresh weight (mg/ seedling)	Dry weight (mg/ seedling)
Cultivars					
Töre	97.5[a†]	3.56[c]	10.74[a]	130.73[bc]	13.30[bc]
Taşkent	81.0[b]	4.32[b]	8.78[bc]	120.67[cd]	12.30[c]
Özkaynak	80.2[bc]	4.12[b]	9.33[b]	115.91[d]	12.34[c]
Ulubatlı	77.0[cd]	4.73[a]	7.91[c]	132.27[b]	13.92[b]
Ürünlü	86.5[bc]	4.15[b]	9.46[b]	140.14[ab]	13.85[b]
Gölyazı	72.8[d]	4.62[a]	8.77[bc]	145.70[a]	15.60[a]
Droughts (bar)					
0 (control)	98.75[a]	2.62[c]	13.38[a]	249.21[a]	22.04[a]
-2	95.83[b]	4.15[b]	8.89[b]	95.17[b]	11.47[b]
-4	52.91[c]	5.98[a]	5.22[c]	48.32[c]	7.15[c]
Analysis of variance					
Cultivars (A)	**	**	**	**	**
Droughts (B)	**	**	**	**	**
A x B	**	**	*	**	**

*, **: significant level of 5% and 1%, respectively. †: letters show different groups at 5% level.

The Töre cultivar achieved the fastest germination, whereas the Ulubatlı and Gölyazı cultivars germinated the slowest (Table 3). Mean germination time was prolonged in all cultivars as drought stress increased (Table 4). The fastest-germinating cultivars at -4 bar of drought stress were the Töre and Ürünlü cultivars. Such findings regarding prolonged mean germination time due to increased drought stress align with the results of Okçu et al. (2005) and Aslan and Atış (2018).

The longest seedlings, at 14.55 cm, were obtained in the control condition of the Töre cultivar, whereas the shortest ones, at 3.96 cm, emerged in the -4 bar application of the Ulubatlı cultivar (Table 4). As drought stress intensified, seedling length steadily decreased in all cultivars. The lowest reduction rates in seedling lengths at -2 bar were recorded in the Töre, Özkaynak, and Ulubatlı cultivars (Figure 3). Although the Ürünlü, Özkaynak, and Töre cultivars showed the lowest reduction rates at -4 bar, the highest reduction rates were observed in the Taşkent, Ulubatlı, and Gölyazı cultivars. Both Okçu et al. (2005) and Petrović et al. (2016) have reported that increased doses of different applications of osmatic pressure created using NaCl and PEG negatively affected root and shoot length in pea genotypes. Added to that, Pereira et al. (2020) found that a reduction in epicotyl and root length in pea genotypes occurred when osmotic potential was no more than 0.2 MPa.

The fresh and dry weights of the seedlings steadily decreased as drought stress increased (Table 4). The highest and lowest fresh and dry weights were obtained for the Gölyazı cultivar in the control condition and in the Taşkent cultivar at -4 bar of drought stress, respectively. The lowest reduction rates in fresh weight at -2 bar of drought stress were recorded for the Töre and Özkaynak cultivars, which paralleled the reduction rates in seedling length (Figure 4). Although the reduction rates of cultivars at -4 bar of drought stress were similar, as with the reduction rates of seedling length, the Töre, Özkaynak, and Ürünlü cultivars had low reduction rates, whereas the Taşkent, Ulubatlı, and Gölyazı cultivars had high ones. Okçu et al. (2005), Petrović et al. (2016), and Pereira et al. (2020) all observed a remarkable decrease in the fresh and dry weights of pea seedlings due to the decreased availability of water as doses of NaCl and PEG increased.

Table 4. The effect of cultivars x droughts interaction on germination and seedling growth characters of forage pea cultivars.

Cultivars	Droughts (bar)	Germination (%)	Mean germination time (day)	Seedling length (cm)	Fresh weight (mg/seedling)	Dry weight (mg/seedling)
Töre	0 (Control)	100.0	2.38	14.55	229.75	20.25
	-2	100.0	3.05	11.28	111.40	12.17
	-4	92.5	5.26	6.38	51.05	7.50
Taşkent	0 (Control)	98.5	2.71	14.20	242.00	21.00
	-2	99.0	4.02	8.05	85.67	10.77
	-4	45.5	6.25	4.10	34.35	5.12
Özkaynak	0 (Control)	98.0	2.36	12.89	206.25	18.50
	-2	97.0	3.84	9.38	89.10	11.22
	-4	45.5	6.15	5.71	52.37	7.30
Ulubatlı	0 (Control)	99.5	3.04	11.81	256.75	23.50
	-2	93.0	4.65	7.95	93.62	11.47
	-4	38.5	6.52	3.96	46.42	6.80
Ürünlü	0 (Control)	96.5	2.71	13.34	263.50	22.00
	-2	96.0	4.18	8.34	97.95	10.85
	-4	67.0	5.55	6.75	58.97	8.70
Gölyazı	0 (Control)	100.0	2.52	13.46	297.00	27.00
	-2	90.0	5.18	8.37	93.32	12.32
	-4	28.5	6.17	4.48	46.77	7.50
$LSD_{\%5}$		6.39	0.46	1.63	18.07	1.76

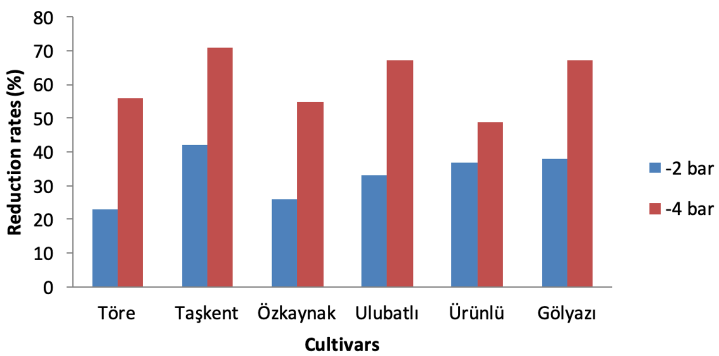

Figure 3. Reduction rates in seedling lengths of forage pea cultivars at -2 and -4 bar of drought stresses

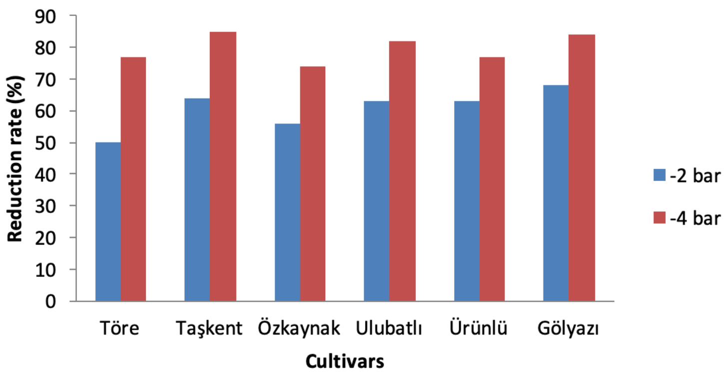

Figure 4. Reduction rates in seedling fresh weights of forage pea cultivars at -2 and -4 bar of drought stresses

Conclusion

In sum, the responses of the cultivars to salinity and drought stresses were similar. The Töre cultivar had high values in terms of germination percentage, mean germination time, and seedling length, whereas the Gölyazı cultivar had high values in the fresh and dry weights of its seedlings in both stress conditions. Depending on the intensity of the stress conditions, reduction rates in the seedling length and fresh weight of the cultivars indicated that the Töre, Özkaynak, and Ulubatlı cultivars were less affected than the Taşkent, Gölyazı, and Ürünlü ones at -2 bar of drought stress and in 15 dS/m of salinity. However, all cultivars were adversely affected in 20 dS/m of salinity and exhibited approximately similar reduction rates. Among other results, as osmotic pressure rose, the Ürünlü cultivar had the lowest reduction rate at -4 bar of drought stress, followed by the Özkaynak and Töre cultivars. The most negatively affected cultivars at that level of drought stress were the Taşkent, Ulubatlı, and Gölyazı ones. Altogether, the results revealed that the Töre cultivar can best tolerate adverse stress conditions but that the Özkaynak cultivar shows promise as well. Although parameters other than reduction rates were disappointing, it can also be focused on the Ulubatlı and Ürünlü cultivars.

Compliance with Ethical Standards

Conflict of interest

The authors declare that for this article they have no actual, potential or perceived the conflict of interests.

Author contribution

The contribution of the authors is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

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