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Effect of Salt Doses on Biological Values in Durum Wheat

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ABSTRACT

This research was carried out to determine the response of some durum wheat cultivars to salt stress during the germination stage. In the study, in which 11 durum wheat varieties, which are widely produced in different regions of Turkey, were used, 3 g l^{-1} and 6 g l^{-1} doses of NaCl were applied in addition to the distilled water application. In the research, germination rate, germination power, root length, shoot length, fresh and dry weight properties were investigated. The study was carried out according to the Factorial Experiment Design in Random Plots under laboratory conditions. Statistically significant differences were found between cultivars and salt doses, except for fresh and dry weight, in terms of all traits examined. Additionally, variety x salt dose interactions were found as important in terms of other characteristics except for dry weight. The highest germination rate, germination power and dry weight were determined in Altıntaş-95, highest root length in Eminbey and highest fresh weight Yelken varieties. Except for and dry weight, other properties were significantly reduced with increasing salt dose.

1. Introduction

Wheat is the plant with the largest cultivation area among the cultivated plants grown for nutritional purposes in the world. It is used as a raw material for foods with high nutritional value such as durum wheat, bulgur and pasta among wheat species, cheap and widely used. In Turkey, which is an important durum wheat producer with Southeastern Anatolia and Central Anatolia regions, durum wheat production has reached approximately 3.15 million tons (Anonymous, 2020).

It is known that salinity is one of the most important abiotic stress factors reducing productivity in agricultural areas, and high salt concentration has a negative effect on seed germination and seedling formation (Fowler, 1991). Plants can easily absorb the soluble salt in the soil. Salt compounds entering the body of the plant show negative effects when they exceed a certain density. With the increase of salt concentration in the soil, it becomes difficult for the plant to take water from the soil, the structure of the soil deteriorates, and plant growth slows down or even stops (Kanber et al., 1992; Güngör and Erözel, 1994).

The negative effect of salinity is seen in more than 20% of agricultural areas in the world (Hafeez et al., 2021). In addition, the rehabilitation of saline areas is very expensive and it cannot be expected to be a permanent solution unless the factors causing salinity are eliminated. For this reason, the determination of salt-resistant varieties has an economic importance in terms of growing plants in these areas. In terms of salt tolerance, there are differences between families, genera and species as well as among varieties belonging to the same species (Kızılgeçi and Yıldırım, 2014).

Plants are more sensitive to salt in the early stages of their development than in other stages of development (Budaklı Çarpıcı and Doğan, 2015). For this reason, it is appropriate to carry out studies in which tolerance to salinity is determined at early developmental stages. The amount of salt present in the environment has a significant effect on the germination, healthy emergence and survival of the seed (Baldwin et al., 1996). Begum et al. (1992) reported that It is a fast and effective technique for determining salt-tolerant wheat varieties, applying salt to seeds during germination and early seedling development.

The high salt levels in the soil and the quality of the irrigation water used are among the factors of concern for future agriculture. Therefore, it is necessary to develop effective strategies to increase yields through salt tolerance (Salim and Raza 2020). The aim of this study is to determine the salt stress tolerance of some durum wheat cultivars grown in different regions of Turkey and to shed light on the studies on salt tolerance.

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2. Material and Method

In the research, 11 durum wheat varieties, which are widely produced in different regions of Turkey, were used. Seeds of the cultivars were produced in Konya ecological conditions during the 2020-2021 cultivation period. The research was carried out in the laboratory of the Department of Field Crops, Faculty of Agriculture, Selcuk University.

NaCl form, which is the most abundant in the soil, accumulates in the soil and affects the cultivated plants the most, was used as salt (Munns and Termaat, 1986). Accordingly, in which distilled water was used as a control, two different NaCl doses, 3 and 6 g l^{-1}, were applied to the seeds. Seeds were left to germinate in dark conditions at 24±1°C in the climate chamber. Before germination, the seeds were subjected to surface sterilization with 1% hypochlorite solution. The seeds were first kept in a solution containing hypochlorite (5% commercial bleach) for 15 minutes and then rinsed three times with sterile distilled water. 5 ml of each application was added to petri dishes with filter paper. 15 seeds were placed in petri dishes and covered with a second filter paper, 3 ml more was added from each application. The data on the examined features were obtained by measuring and counting on the 4th and 8th days in accordance with the ISTA criteria. Seeds exceeding 2 mm in rootlet length were considered as germinated and counted (Fuller et al., 2012). The "germination rate" was determined by counting the seeds germinated on the 4th day, and "germination power", "root length", "shoot length", "fresh weight" and "dry weight" were determined by counting and measurements made on the 8th day. While determining these characteristics, 10 seedlings randomly selected from each petri dish were used. Dry weight was determined by drying fresh shoots at 70°C for 24 hours (Atak et al., 2006; Saboora et al., 2006).

The data obtained from the experiment were subjected to statistical analysis using the JUMP-13 package program in accordance with the Factorial Experiment Design in Random Plots, and the mean values that were significant in the F test were divided into groups with the Student-t test.

3. Results and Discussion

The variance analysis results regarding the germination rate, germination power, root length, shoot length, fresh weight and dry weight characteristics of the wheat varieties used in the present research are given in Table 1.

Table 1
Variance analysis results of traits of durum wheat varieties examined at different salt doses

		F Value					
SOV	DF	Germination Rate	Germination Power	Root Length	Shoot Length	Fresh Weight	Dry Weight
Variety	10	7.51**	6.47**	11.08**	8.10**	8.00**	3.10*
Salt	2	|218.96**	115.78**	161.43**	288.02**	1.92	0.73
Variety x Salt	20	4.99**	3.84**	7.33**	3.84**	4.57**	1.25
Error	64	-	-	-	-	-	-
Total	98	-	-	-	-	-	-

Germination Rate and Power

According to the obtained results, statistically significant differences at the level of 1% were found between varieties and salt doses in terms of germination rate and germination power. In addition, the variety x salt interaction was found to be significant at the 1% level (Table 1).

The highest germination rate and power were determined in Altıntaş-95 variety. This was followed by Eminbey and Ç-1252 varieties, respectively. The lowest germination rate and power were determined in Svevo and Yelken cultivars (Table 2 and Table 3). The difference between the cultivars showing the highest and lowest germination rate and power was 19.3% and 24.8%, respectively. According to the findings of this study, the germination power values of the cultivars were largely parallel to the germination rate values. In terms of both characteristics, the cultivars in the first place and the cultivars in the last place were the same. In addition, in the multiple comparison test, groups were largely similar in terms of both characteristics. These differences between cultivars under laboratory conditions where all conditions are equal can be explained by the difference in salt sensitivity of genotypes.

While the highest germination rate and power values in all cultivars used were determined in the control application, significant decreases occurred in both traits as the salt doses increased (Table2 and Table 3). As the mean values of the cultivars, the germination rate in the control was 87.6% and the germination power was 94.5%, while the related characteristics were determined as 75.3% and 80.7% at 3 g l^{-1} salt dose, 54.0% and 65.0% at 6 g l^{-1} salt dose, respectively. According to these results, it can be said that increasing salt doses negatively affect embryo growth. Benlioğlu and Özkan (2015) state that this may be due to the decrease in the efficiency of water in salty environments, as well as the decrease in the transport of water and storage foodstuffs.

A previous study of Ekmekçi et al. (2005) stated that the decrease in germination rate at increasing salt levels is due to the toxicity of Na+ and Cl- ions, as well as the increasing osmotic pressure preventing the seed from taking up the water required for germination. Decreased germination rate and power at high salt doses may also be associated with higher cell membrane damage (Alamri et al., 2020). It has also been found in other studies that as the salt doses increase, the germination rate and germination power decrease (Huang and Redmann 1995; Pancholi et al., 2001; Prazak, 2001; Şenay et al., 2005; Kara et al., 2011; Benlioğlu and Özkan 2015).

Table 2
Mean values for germination rate of durum wheat varieties at different NaCl doses and multiple comparison results

	Germination Rate (%)			
Variety / Salt Dose	0	3 g l^{-1}	6 g l^{-1}	Mean
Altıntaş-95	95.5 a	82.2 cde	69.9 fgh	82.6 a
Ç-1252	86.6 a-d	82.2 cde	57.8 ıj	75.5 bc
Dumlupınar	83.3 b-e	64.4 hı	56.6 ıj	68.1 de
Eminbey	93.3 ab	91.1 abc	66.6 ghı	81.4 ab
Kunduru-1149	86.6 a-d	91.1 abc	39.9 l	72.5 cd
Kızıltan-91	91.1 abc	84.4 b-e	44.4 kl	73.3 cd
Mirzabey-2000	82.2 cde	80.0 def	43.3 kl	68.5 de
Soylu	88.9 a-d	75.5 efg	53.3 jk	72.6 cd
Svevo	83.3 b-e	62.2 hıj	44.4 kl	63.3 e
Türköz	91.1 abc	68.9 gh	57.7 ıj	72.5 cd
Yelken	82.2 cde	53.3 jk	60.0 hıj	65.1 e
Mean	87.6 a	75.3 b	54.0 c	

Table 3
Mean values for germination power of durum wheat cultivars at different NaCl doses and multiple comparison results

	Germination Power (%)			
Variety / Salt Dose	0	3 g l^{-1}	6 g l^{-1}	Mean
Altıntaş-95	97.8 ab	93.3 a-d	93.3 a-d	91.4 a
Ç-1252	93.3 a-d	86.6 b-f	83.3 c-f	81.4 bc
Dumlupınar	93.3 a-d	66.6 g-j	75.5 f-ı	78.5 bcd
Eminbey	100.0 a	88.8 b-f	68.8 g-j	85.9 ab
Kunduru-1149	95.5 abc	91.1 a-d	56.6 jkl	81.1 bc
Kızıltan-91	95.5 abc	86.6 b-f	48.9 kl	77.0 cd
Mirzabey-2000	97.8 ab	88.8 a-e	76.6 e-h	84.0 bc
Soylu	97.8 ab	82.2 def	57.7 jkl	79.2 bcd
Svevo	91.1 a-d	62.2 j	46.6 l	66.6 e
Türköz	95.5 abc	77.7 efg	76.6 e-h	83.3 bc
Yelken	93.3 a-d	63.3 ıj	60.0 jk	72.2 de
Mean	94.5 a	80.7 b	65.0 c	

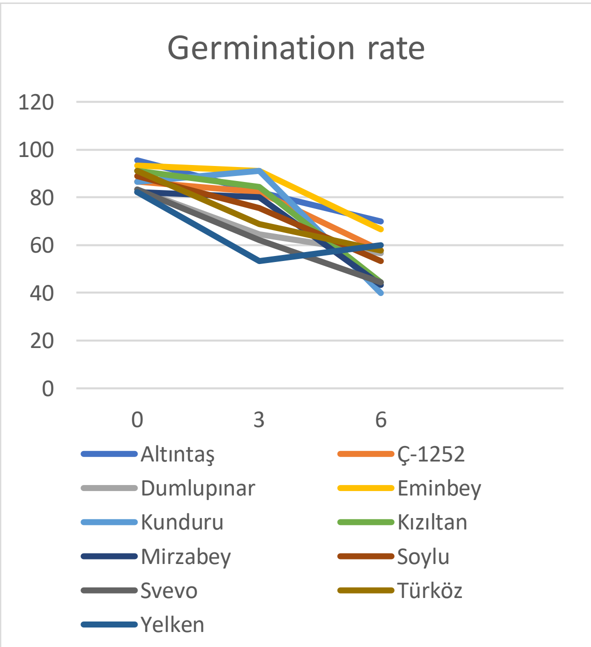

Figure 1
Interaction (variety x salt) graph of germination rate values of durum wheat varieties

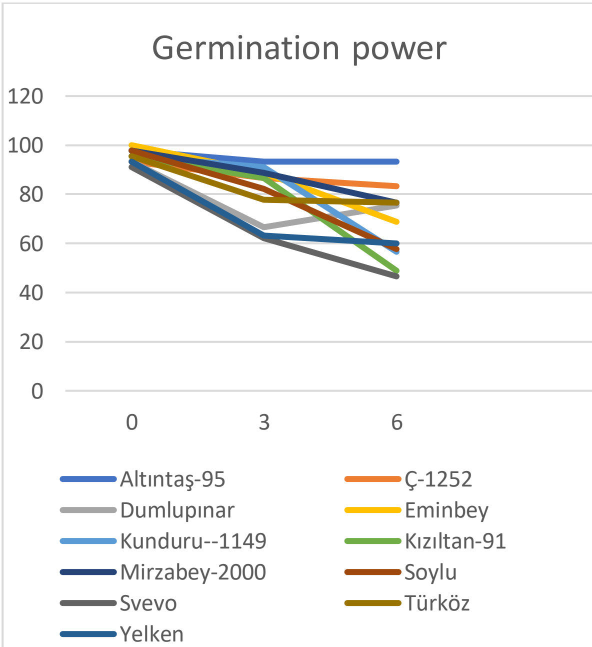

Figure 2
Interaction graph of germination power values of durum wheat varieties (variety x salt)

Present research showed that, the variety x salt interaction was found to be important in terms of germination rate and power. This is due to the different responses of cultivars to different salt doses. As a matter of fact, while the germination rate values of Kunduru-1149 cultivar were 86.6% in the control, it increased to 91.1% with an increase of 4.5% at the 3 g l^{-1} salt dose and decreased to 39.9% at 6 g l^{-1} dose. On the other hand, while the germination rate in Yelken variety was 82.2% in the control application, it decreased to 53.3% with a 28.9% decrease at 3 g l^{-1} salt dose, and increased to 60.0% with an increase of 6.7% at 6 g l^{-1} salt dose (Figure 1). Except for Kunduru-1149 and Yelken, the highest germination rate was determined in the control application, the germination rate values decreased regularly depending on the increasing salt dose, and the lowest values were determined at the highest salt dose.

In eight cultivars except Altıntaş-95, Dumlupınar and Türköz, the highest germination power values were obtained from the control application, and the germination power decreased significantly depending on the increasing salt dose (Figure 2). On the other hand, while the highest values were obtained from control in Altıntaş-95 and Türköz cultivars, germination power remained constant in both cultivars at 3 and 6 g l^{-1} salt doses; In the Dumlupınar variety, the germination power, which was 66.6% at 3 g l^{-1}, increased to 75.5% at 6 g l^{-1} salt dose. The results indicated that, the different response of cultivars to salt doses in terms of germination rate and germination power can be explained by the change in physiological characteristics of the cultivars depending on their genetic structures.

Root and Shoot Length

According to the results, statistically significant differences at the level of 1% were found between varieties and salt doses in terms of root and shoot length. In addition, the variety x salt interaction was found to be significant at the 1% level (Table 1).

The highest root length was determined in Eminbey variety, followed by Kunduru-1149, Kızıltan-91 and Altıntaş-95 varieties. The lowest root length was determined in Mirzabey-2000 and Türköz cultivars, respectively (Table 4). Additionally, the shoot length values of the cultivars were generally lower than the root length values. The highest shoot length was determined in Ç-1252 cultivar, followed by Dumlupınar cultivar, the lowest shoot length was determined in Kunduru-1149 and Svevo cultivars (Table 5).

Considering that all conditions are equal, the different performances of the cultivars in terms of root and shoot length can be explained by the change in the physiological characteristics of the cultivars depending on the genetic structure.

Table 4
Mean values for root length of durum wheat cultivars at different NaCl doses and multiple comparison test results

	Root Length (cm)			
Variety / Salt Dose	0	3 g l^{-1}	6 g l^{-1}	Mean
Altıntaş-95	12.5 bcd	8.9 ı-m	7.0 m-p	9.4 a
Ç-1252	8.2 j-n	12.0 b-e	7.2 m-p	9.1 ab
Dumlupınar	11.6 c-f	6.8 nop	9.2 h-l	9.2 ab
Eminbey	16.0 a	7.3 l-p	6.8 nop	10.0 a
Kunduru-1149	12.7 bcd	10.3 e-ı	5.8 pq	9.6 a
Kızıltan-91	13.9 b	9.5 g-k	6.3 nop	9.9 a
Mirzabey-2000	6.1 opq	7.5 k-p	3.5 r	5.7 c
Soylu	13.6 bc	8.2 j-n	6.5 nop	9.4 ab
Svevo	11.2 d-g	9.7 f-j	6.4 nop	9.1 ab
Türköz	10.5 e-ı	5.8 pq	4.3 qr	6.8 c
Yelken	11.2 d-h	8.0 j-o	5.8 pq	8.3 b
Mean	11.6 a	8.5 b	6.2 c	

Table 5
Mean values for shoot length of durum wheat cultivars at different NaCl doses and multiple comparison test results

	Shoot Length (cm)			
Variety / Salt Dose	0	3 g l^{-1}	6 g l^{-1}	Mean
Altıntaş-95	8.2 bc	6.6 d-g	4.4 h-k	6.4 de
Ç-1252	9.4 ab	9.4 ab	5.6 ghı	8.1 a
Dumlupınar	9.4 ab	7.6 cde	5.8 fgh	7.6 ab
Eminbey	10.4 a	4.0 jkl	3.7 klm	6.0 ef
Kunduru-1149	8.0 bcd	5.5 g-j	2.5 lm	5.3 f
Kızıltan-91	10.0 a	7.9 b-e	3.5 klm	7.1 bcd
Mirzabey-2000	10.5 a	6.5 efg	4.2 ıjk	7.1 bcd
Soylu	10.4 a	6.4 efg	3.2 klm	6.6 cde
Svevo	6.9 c-g	6.4 efg	2.2 m	5.3 f
Türköz	10.2 a	7.2 c-f	3.5 klm	7.0 bcd
Yelken	10.4 a	7.7 cde	4.0 jkl	7.3 abc
Mean	9.4 a	6.9 b	3.9 c	

Considering the mean values of 11 cultivars used in the study, the highest root and shoot length values were determined in the control application, while significant decreases occurred in both characteristics as the salt doses increased. While the mean values of the cultivars were 11.6 cm root length and 9.4 cm shoot length in the control, the relevant characteristics were determined as 8.5 cm and 6.9 cm at 3 g l^{-1} salt dose, 6.2 cm and 3.9 cm at 6 g l^{-1} salt dose, respectively. These results show that increasing salt doses negatively affect root and shoot length. In many studies on the subject, it has been stated that root length is significantly affected by salt stress (Jamil et al., 2005; Dumlupınar et al., 2007; Jafarzadeh and Aliasgharzad 2007; Benlioğlu et al., 2015).

In salty conditions, the hormones in the plant are negatively affected, as a result, while the cytokinin hormone, which promotes cell division and shoot development, decreases; On the other hand, the amount of abscisic acid (ABA) increases, which prevents early germination of seeds and ensures the closure of stomata (Güneş et al., 2007; Kumlay and Eryiğit 2011). Similarly, plant growth slows down due to Na toxicity in salty environments, and nutrient intake, especially calcium and potassium, decreases by causing both osmotic and ionic stresses (Zahra et al., 2018; Salim and Raza 2020).

Root development is one of the important indicators of salt resistance (Janmohammadi et al., 2012). Khan et al. (2003) stated that root length is an important parameter that can be used in the selection of salt-tolerant genotypes. During germination, if there is no salt barrier in water intake, rootlets develop normally, otherwise, rootlet development decreases due to salt stress (Oyiga et al., 2016).

According to the results of the present research, it was determined that the shoot lengths were shorter than the root lengths. Güneş et al. (2007) reported that the shoot length was more affected by the root length at increasing salt doses, which may be related to the change in the water status of the leaves while Salim and Raza (2020) reported that, the decrease in the water content of the leaves negatively affects photosynthetic activity and protein synthesis. The fact that shoot growth is more affected by root growth in saline environments can also be considered as a result of the effort to reduce the water requirement of the plant. Oyiga et al. (2016) stated that, in salty environments, a lower nutrient uptake occurs and the contribution of carbohydrates to young leaves is reduced.

Results of the present research revelaed that, it was determined that root and shoot length also changed significantly depending on the variety and salt concentration (Table 1). As a matter of fact, while the root length of Ç-1252 cultivar was 8.2 cm in the control, it increased to 12.0 cm with an increase of approximately 50% at a salt dose of 3 g l^{-1}, and decreased by approximately 41% at 6 g l^{-1} dose Similarly, while Mirzabey-2000 cultivar had a root length of 6.1 cm in salt-free environment, the root length increased to 7.5 cm at 3 g l^{-1} salt dose, and decreased to 3.5 cm with a decrease of more than 100% at 6 g l^{-1} dose. While the root length was 11.6 cm in the control application of Dumlupınar cultivar, it decreased to 6.2 cm at 3 g l^{-1} salt dose, and increased by 35% at 6 g l^{-1} to 9.2 cm. On the other hand, in eight cultivars other than these three, the highest values were obtained in the control and root lengths decreased significantly depending on the increasing salt dose (Figure 3).

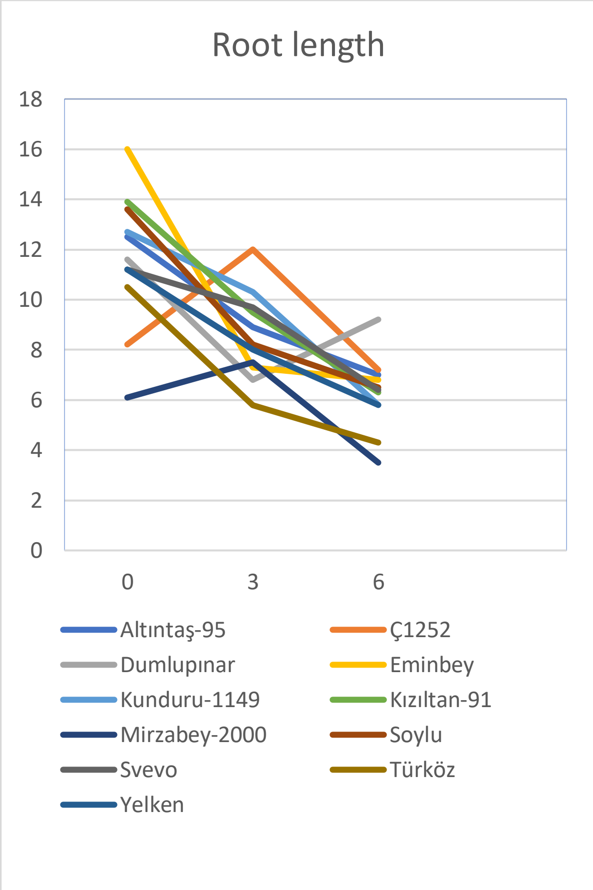

Figure 3
Interaction graph of root length values of durum wheat cultivars (variety x salt)

On the other hand, Ç-1252 and Eminbey cultivars reacted differently to increasing salt doses in terms of shoot length than other cultivars. For example, while shoot length was constant (9.4 cm) in control and 3 g l^{-1} salt dose in Ç-1252 variety, it was found close to each other (4.0 and 3.7 cm, respectively) in Eminbey variety at 3 g l^{-1} and 6 g l^{-1} doses. In the other nine cultivars, shoot length was the longest in the control, while shoot lengths decreased significantly depending on the increasing salt dose (Figure 4). The difference in root and shoot lengths of genotypes according to salt concentration can be explained by the change in their physiological characteristics depending on the genetic structure of the cultivars.

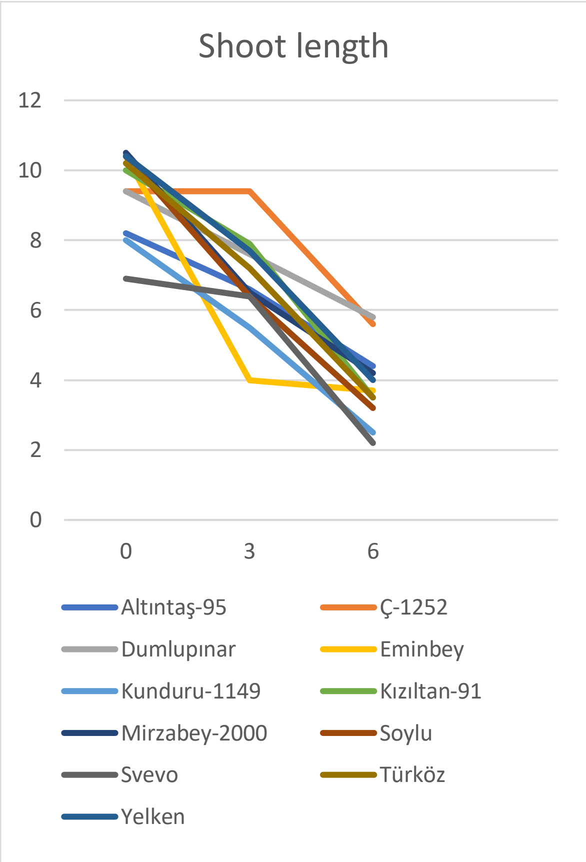

Figure 4
Interaction (variety x salt) graph of shoot length of durum wheat cultivars

Fresh Weight and Dry Weight

Analysis of variance revealed that, significant differences were determined between the varieties in terms of fresh weight at the level of 1% and in terms of dry weight at the level of 5%. In addition, the variety x salt interaction in terms of fresh weight was found to be significant at the 1% level (Table 1).

In the present research, the highest fresh weight was found in Yelken variety with 1.4 g, and the highest dry weight was determined in Altıntaş-95 variety with 0.60 g. The lowest fresh weight was determined in Dumlupınar, Soylu and Svevo, and the lowest dry weight was determined in Mirzabey-2000 and Svevo varieties (Table 6 and Table 7). In terms of dry weight, except Altıntaş-95 variety, the varieties in the first row and the varieties in the last row were found to be approximately the same in terms of both characteristics. These differences between varieties under laboratory conditions where all conditions are equal can be explained by the difference in salt sensitivity of genotypes.

Table 6
Mean values for fresh weight (g) of durum wheat varieties at different NaCl doses and multiple comparison test results

	Fresh Weight (g)			
Variety / Salt Dose	0	3 g l^{-1}	6 g l^{-1}	Mean
Altıntaş-95	1.2 b-ı	1.3 a-f	0.9 ı-m	1.1 bcd
Ç-1252	0.8 j-n	1.5 abc	1.4 a-e	1.3 abc
Dumlupınar	0.6 l-o	0.5 no	1.3 a-g	0.8 e
Eminbey	1.6 a	1.4 a-f	0.9 g-m	1.3 abc
Kunduru-1149	1.2 b-ı	1.3 a-f	0.6 l-o	0.9 de
Kızıltan-91	1.0 f-l	1.4 a-f	1.1 d-k	1.2 bc
Mirzabey-2000	0.8 k-o	1.2 b-j	1.2 a-ı	1.1 cd
Soylu	0.9 g-m	0.6 mno	1.1 e-k	0.8 e
Svevo	0.4 o	1.1 c-k	0.9 h-m	0.8 e
Türköz	1.4 a-e	1.3 a-g	1.2 a-h	1.3 ab
Yelken	1.3 a-f	1.5 a-d	1.5 ab	1.4 a
Mean	1.0	1.1	1.1	

Table 7
Mean values for dry weight (g) of durum wheat varieties at different NaCl doses and multiple comparison test results

	Dry Weight (g)			
Variety / Salt Dose	0	3 g l^{-1}	6 g l^{-1}	Mean
Altıntaş-95	0.54	0.64	0.62	0.60 a
Ç-1252	0.32	0.35	0.38	0.35 bcd
Dumlupınar	0.35	0.30	0.27	0.30 d
Eminbey	0.47	0.52	0.29	0.43 bcd
Kunduru-1149	0.26	0.32	0.32	0.30 d
Kızıltan-91	0.38	0.29	0.28	0.32 cd
Mirzabey-2000	0.27	0.29	0.28	0.28 d
Soylu	0.32	0.35	0.38	0.35 bcd
Svevo	0.30	0.26	0.28	0.28 d
Türköz	0.36	0.46	0.35	0.39 bcd
Yelken	0.48	0.45	0.53	0.48 ab
Mean	0.41	0.38	0.36	

It was determined that there was no significant decrease in the fresh and dry weight values with the increase of the salt dose. Although the shoot and root length values decreased significantly due to the increasing salt dose, the lack of a significant change in the fresh and dry weight values may be due to the insufficient use of storage nutrients in the seeds during germination at high salt concentrations (Benlioğlu and Özkan 2015). Unlike the results which obtained from the present study, in some studies conducted on the subject, it was determined that the dry weights of the varieties showed higher values as the salt dose increased (Sultana et al., 2000; Benlioğlu and Özkan 2015).

Present research showed that, the responses of cultivars to salt concentrations in terms of fresh weight were also different. For example, while the fresh weight values of Dumlupınar, Eminbey, Soylu and Türköz cultivars decreased at different rates at a salt dose of 3 g l^{-1} compared to the control, on the contrary, it increased at different rates in other cultivars (Figure 5).

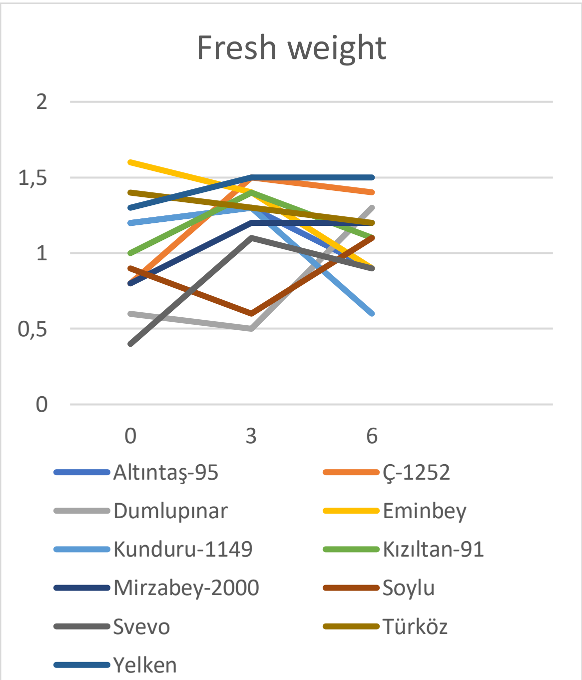

Figure 5
Interaction graph of fresh weight values of durum wheat varieties (variety x salt)

4. Conclusion

In this study, in which the responses of 11 durum wheat varieties, which are widely produced in Turkey, to different salt doses during the germination period were investigated, significant differences were found between the varieties in terms of all the examined characteristics. The highest germination rate and germination power were determined in Altıntaş-95 variety. This was followed by Eminbey and Ç-1252 varieties, respectively. The lowest germination rate and power were determined in Svevo and Yelken cultivars. In addition, the highest root length was determined in Eminbey variety, followed by Kunduru-1149, Kızıltan-91 and Altıntaş-95 varieties. The lowest root length was determined in Mirzabey-2000 and Türköz cultivars, respectively. Shoot length values were generally lower than root length values. The highest shoot length was determined in Ç-1252 cultivar, followed by Dumlupınar cultivar, the lowest shoot length was determined in Kunduru-1149 and Svevo cultivars. In addition to these findings, the highest fresh weight was found in Yelken variety with 1.4 g, and the highest dry weight was determined in Altıntaş-95 variety with 0.60 g. The lowest fresh weight was determined in Dumlupınar, Soylu and Svevo, and the lowest dry weight was determined in Mirzabey-2000 and Svevo varieties.

It was observed that with the increase of salt concentration, both the germination rate decreased and the germination was significantly delayed. Root and shoot lengths, which are important features in determining the salinity tolerance of genotypes, were found to decrease significantly with the increase in salt concentration. In the study, it was determined that the responses of the varieties to salt doses also changed in terms of other characteristics except dry weight.

According to the results obtained from the study, Altıntaş-95, Kunduru-1149, Mirzabey and Ç-1252 varieties can be recommended welded by germination power in conditions where salt concentration is high. It would be appropriate to propose a variety for salty soils after a study to be carried out to cover the germination and seedling periods.

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