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DETERMINATION OF SALT TOLERANCE OF SOME ANNUAL GRASS TYPES UNDER IN VITRO CONDITIONS

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Abstract
Salinity effects in this period may cause insufficient development of the plant in the later periods. For the continuation of plant production in salinized areas, it is necessary to determine and increase the salt resistance of genotypes. Annual grass plants are grown extensively in the world. However, as with other plant species, annual grass plants are adversely affected by soil salinity. The aim of present study; determination of the salt tolerance of annual grass cultivars in vitro. In the study, 25 registered grass varieties were used as material. Salt concentrations of 150 mM NaCl were applied to determine the response of annual grass varieties to salt stress. Root length, shoot length, root-shoot fresh weight and root-shoot dry weight were examined in in vitro salt testing and the lowest values in the control group in terms of these properties were obtained from Efe and Vallivert cultivars, while the highest value was obtained from Medoacus and Trinova cultivars. In salt application, the lowest value was obtained from Tornado, Vallivert, Venus varieties, while the highest value was obtained from Medoacus and Trinova varieties. In addition, the variation obtained among the annual grass varieties used in the study can be used both in the grass aplication and in breeding programs.

Keywords: Annual grass, salt stress, in vitro testing

1. INTRODUCTION

In terms of animal nutrition and use in landscaping, annual grass plants are a tall forage plant that can grow fast and can take more than one form under suitable climate and soil conditions. Annual grass plant; In terms of its agricultural characteristics, it is a forage plant that has increased rapidly in field agriculture in recent years and has the potential to increase due to its rapid development, abundant grass production, positive response to fertilizers in irrigated conditions, taking place in crop rotation and being a single year (Taşsever, 2019).

In order to be successful in field agriculture, it is of great importance to choose the appropriate variety, to know where and how to grow it (Arslan ve Çakmakçı, 2004). Because each variety has good and bad features, strengths and weaknesses. These characteristics should be well known in breeding conditions. Because, as well as yield and quality characteristics, the compatibility of varieties and regional conditions is of great importance in terms of yield and quality characteristics. The newly developed cultivars for this purpose differ considerably according to the salt tolerance level.

Salinity; it is the event that soluble salts, which are washed and mixed with groundwater, come to the soil surface through capillarity with high ground water and as a result of evaporation, the water leaves the soil and accumulates on the soil surface and near the surface, particularly in arid and semi-arid climatic regions (Kara, 2002). Soluble salts can be easily taken up by plants. Depending on the type

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and amount of salt compounds entering the plant, they become harmful to the plant when they exceed a certain concentration. They have a toxic effect on the plant by disrupting nutrition and metabolism. In addition, when the salt concentration soil increased, it becomes difficult to take water from the soil for the plant, the structure of the soil gey worse, and plant growth slows down or even stops(Kanber et al. 1992; Güngör and Erözel, 1994). The tolerance of plants to salt is closely related to the development period. In general, all plants are more sensitive to salt in the germination and early development stages. Salinity effects in this period may cause insufficient development of the plant in the later periods.
For the continuation of plant production in salinized areas, it is necessary to determine and increase the salt resistance of genotypes. Today, classical breeding methods can be supported by in vitro techniques, and salt-tolerant cells can be selected in the culture medium and plants can be obtained from them (Tal 1994, Winicow 1996). The germination test, which is evaluated with different physiological and biochemical parameters, determines the salt tolerance of plants during the germination and seedling periods(Soltani et al. 2004, Benlioğlu and Özkan, 2015, Sultana et al. 2022, Liu et al. 2010,). Annual grass plants are grown extensively in the world. However, as with other plant species, annual grass plants are adversely affected by soil salinity. Preventing salinization in the open field is both expensive and laborious. Although production with salt-tolerant varieties is the most applicable method, it is very important to know the salt tolerance levels of the varieties and to decide on the type or variety to be grown according to the salinity level determined by soil analysis. For these reasons, in present study, it was aimed to determine the tolerance levels of 25 different annual grass varieties against salinity *in vitro*.

2. MATERIALS AND METHODS

In the present study, 25 different annual grass varieties registered in Turkey (Vallivert, Master, Hellen, Venus, Excellent, BigBoss, Jivet, Cesco, Trinova, Tornado, First Step, Ration, Quickston, Jako, Elif, Braulio, Baqueno, Kar Tetra, Devis) , Rambo, Teanna, Efe 82, Medoacus, Caramba, Koga) were used as plant material. In the study, MS(hormone free)+3% sucrose+0.7% plant agar was added in control applications, and MS(hormone free)+3% sucrose+0.7% plant agar+150 mM NaCl was added in NaCl applications. The prepared media were autoclaved at 122℃ for 20 minutes. Seeds of annual grass genotypes were soaked in 70% alcohol for 1 minute in a sterile cabinet, then surface-sterilized by soaking the seeds in 10% hypo cloride for 15 min and rinsed 3 times with sterile distilled water. Seed sowing was done in small glass jars with the medium poured in, with 10 seeds in each jar. Eight replications of each variety were used, including 4 control and 4 application jars. The jars prepared for germination were kept in a plant growth room at 25±2℃ and 3000-4000 lux for 8 days. Measurements were made 8 days after sowing. Root length (cm), shoot length (cm), root + shoot fresh weight (mg), root + shoot dry weight (mg) were determined in the application and control plants. Result of the research were analyzed by using the SAS (SAS Inst. 1999) package program according to the split plot design in random plots. Whether the difference between the found averages was significant or not was determined by the LSD test.

3. RESULTS AND DISCUSSIONS

3.1. Root Length(cm)

Root length values of different grass varieties under control and salt-free stress in vitro conditions are given in Table 3.1. The effect of salt application and cultivars on root length was statistically very significant($p \leq 0.01$). Root length varied between 0.94-4.11 cm in the control application. The lowest

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root length was obtained from Efe 82 variety, and the highest value was obtained from Medoacus annual grass variety. Root lengths varied between 0.54-2.43 cm under salt stress. The lowest root length was obtained from Tornado variety, and the highest root length value was obtained from Medoacus variety under salt stress.

Table 3.1. Root lengths of annual grass varieties germinated in vitro under salt application.

Varieties	Control	NaCI (150 mM)
Vallivert	3.04^{A-E}	0.74^{FG}
Master	2.61^{B-F}	0.69^{G}
Hellen	3.25^{A-D}	0.61^{G}
Venus	2.41^{C-F}	0.73^{G}
Excellent	2.62^{B-F}	0.79^{FG}
BigBoss	2.54^{B-F}	0.78^{FG}
Jivet	3.29^{A-D}	0.92^{EFG}
Cesco	1.69^{GH}	0.79^{FG}
Trinova	2.07^{EFG}	0.93^{EFG}
Tornado	2.58^{B-F}	0.54^{G}
İlk Adım	1.67^{GH}	0.69^{G}
Ration	1.95^{FGH}	1.34^{C-F}
Quickston	2.48^{B-F}	1.61^{BCD}
Jako	3.39^{ABC}	1.66^{BC}
Elif	2.81^{B-F}	1.52^{B-E}
Braulio	3.52^{AB}	1.71^{BC}
Baqueno	2.29^{D-G}	2.11^{AB}
Kar Tetra	3.42^{ABC}	1.79^{BC}
Devis	2.68^{B-F}	1.75^{BC}
Rambo	3.46^{ABC}	1.49^{CDE}
Teanna	2.15^{EFG}	1.02^{D-G}
Efe 82	0.94^{H}	0.85^{FG}
Medoacus	4.11^{A}	2.43^{A}
Caramba	3.03^{A-F}	1.87^{ABC}
Koga	1.62^{GH}	0.81^{FG}

3.2. Shoot Length (cm)

Shoot length values of annual grass varieties under salt stress and control application are given in Table 3.2. The effect of salt stress and variety on annual grass shoot length was found to be statistically significant at the 1% level. Salt stress caused shortening of shoot length in all cultivars. In the control application, the lowest root length value was obtained from the variety Efe 82 with 3.05 cm, and the highest shoot length value was obtained from the variety Medoacus with 10.44 cm. In the application of 150 mM NaCI salt stress, the lowest shoot length value was obtained from the Tornado variety with 0.38 cm, and the highest shoot length value was obtained from the Medoacus variety with 4.05 cm.

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Table 3.2. Shoot lengths of annual grass varieties germinated in vitro under salt application.

Varieties	Control	NaCI (150 mM)
Vallivert	5.98^{D-H}	0.65^{HIJ}
Master	4.69^{GHI}	0.52^{IJ}
Hellen	6.05^{D-G}	0.41^{J}
Venus	4.65^{GHI}	0.62^{HIJ}
Excellent	6.29^{C-G}	0.84^{G-J}
BigBoss	4.53^{GHI}	0.49^{IJ}
Jivet	5.74^{E-H}	0.57^{IJ}
Cesco	3.93^{HI}	0.54^{IJ}
Trinova	4.83^{GHI}	0.55^{IJ}
Tornado	5.23^{GH}	0.38^{J}
İlk Adım	3.88^{HI}	0.57^{IJ}
Ration	4.45^{GHI}	1.45^{F-I}
Quickston	4.73^{GHI}	$1.90D^{EF}$
Jako	8.93^{AB}	2.60^{BCD}
Elif	7.79^{B-E}	2.50^{B-E}
Braulio	8.22^{BC}	1.72^{D-G}
Baqueno	6.25^{C-G}	2.95^{BC}
Kar Tetra	8.53^{AB}	2.70^{BCD}
Devis	7.38^{B-F}	1.88^{DEF}
Rambo	7.97^{BCD}	1.32^{F-J}
Teanna	$5.46F^{GH}$	1.09^{F-J}
Efe 82	3.05^{I}	1.56^{E-H}
Medoacus	10.44^{A}	4.05^{A}
Caramba	7.52^{B-F}	3.20^{AB}
Koga	8.00^{BCD}	2.00^{C-F}

3.3. Root + Shoot Fresh Weight (mg)

Average values of root + shoot fresh weight of annual grass varieties are given in Table 3.3. The effect of 150 mM NaCI application and variety on root + shoot fresh weight was found to be statistically significant at the 1% level.

When the root + shoot fresh weight values in control petri dishes and under salt stress were examined, the lowest values were obtained from the Vallivert variety as 13.56 mg and 6.59 mg, respectively, and the highest root + shoot fresh weight values were obtained from the Medoacus one-year grass variety as 37.50 mg and 19.00 mg, respectively.

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Table 3.3. Root + shoot fresh weights annual grass varieties germinated in vitro under NaCI application

Varieties	Control	NaCI (150 mM)
Vallivert	13.56^{K}	6.59^{D}
Master	21.14^{HI}	10.76^{BCD}
Hellen	23.24^{F-I}	10.84^{BCD}
Venus	15.75^{JK}	7.38^{CD}
Excellent	23.19^{GHI}	10.82^{BCD}
BigBoss	24.86^{D-H}	12.17^{BCD}
Jivet	29.96^{BCD}	11.79^{BCD}
Cesco	23.23^{F-I}	10.13^{BCD}
Trinova	26.51^{D-G}	12.56^{BCD}
Tornado	28.00^{C-G}	11.30^{BCD}
İlk Adım	26.34^{K}	26.33^{A}
Ration	24.37^{E-H}	14.34^{BCD}
Quickston	13.28^{K}	10.05^{BCD}
Jako	32.76^{ABC}	18.81^{ABC}
Elif	25.40^{D-H}	14.08^{BCD}
Braulio	33.42^{AB}	18.97^{AB}
Baqueno	28.31^{C-F}	18.33^{ABC}
Kar Tetra	28.37^{B-E}	15.98^{A-D}
Devis	24.84^{E-H}	15.49^{A-D}
Rambo	24.23^{E-H}	12.74^{BCD}
Teanna	25.85^{D-H}	13.76^{BCD}
Efe 82	18.80^{IJ}	13.35^{BCD}
Medoacus	37.50^{A}	19.00^{AB}
Caramba	28.39^{B-E}	18.65^{ABC}
Koga	24.66^{E-H}	12.80^{BCD}

3.4. Root + Shoot Dry Weight (mg)

Root + shoot dry weight values of annual grass varieties grown under salt stress are given in Table 3.4. The effect of NaCI application and variety on root + shoot dry weights was found to be statistically very significant ($p \leq 0.01$). The lowest root + shoot dry weight value was obtained from the Venus variety with 2.16 mg in the control application and 1.62 mg in the NaCI application. The highest root + shoot dry weight value was obtained from Trinova variety with 5.19 mg in control application and 4.55 mg in NaCI application.

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Table 3.4. Root + shoot dry weights of annual grass varieties germinated in vitro under NaCI application.

Varieties	Control	NaCI (150 mM)
Vallivert	3.46^{FG}	3.15^{DE}
Master	4.05^{B-F}	3.90^{ABC}
Hellen	4.41^{A-F}	3.90^{ABC}
Venus	2.16^{H}	1.62^{G}
Excellent	3.48^{FG}	3.28^{B-E}
BigBoss	4.71^{A-D}	4.43^{A}
Jivet	4.65^{A-E}	3.93^{ABC}
Cesco	4.52^{A-F}	3.95^{ABC}
Trinova	5.19^{A}	4.55^{A}
Tornado	4.92^{A-D}	4.36^{A}
İlk Adım	2.36^{GH}	2.26^{FG}
Ration	4.16^{A-F}	3.61^{BCD}
Quickston	2.21^{H}	2.11^{FG}
Jako	3.80^{DEF}	3.58^{BCD}
Elif	3.39^{FG}	2.32^{FG}
Braulio	5.11^{AB}	4.55^{A}
Baqueno	4.43^{A-F}	3.91^{ABC}
Kar Tetra	3.42^{FG}	3.25^{CDE}
Devis	4.37^{A-F}	3.59^{BCD}
Rambo	4.28^{A-F}	3.15^{DE}
Teanna	3.55^{EF}	3.06^{DE}
Efe 82	3.83^{DEF}	1.80^{G}
Medoacus	4.62^{A-E}	4.00^{AB}
Caramba	4.99^{ABC}	3.43^{B-E}
Koga	3.87^{C-F}	2.71^{EF}

It has been reported that there are genetic differences between cultivars in terms of tolerance to salt stress. However, it was determined that the increase in salinity affected all varieties negatively, and some varieties were more tolerant than others (Carpıcı et al. 2009). In our study, the response of cultivars to salt was different. Many studies have reported that genetically varieties respond differently to salt stress. Moud and Maghsoudi (2008) reported that the seedling responses of wheat cultivars were different under salt stress, but there was a decrease in coleoptile and root growth at both salinity levels. They stated that these results showed that there was genetic variation among cultivars in terms of early seedling growth rate under salt stress conditions. They also concluded that coleoptile and root length, as well as germination and emergence rates, could be used as selection criteria for salt stress tolerance at early growth stages. Under salt stress conditions, coleoptile elongation rate decreases due to low soil water potential (Francios et al. 1986), and plant growth

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remains poor due to weak coleoptile and root growth. Huang and Reddman (Huang and Reddman, 1995) in barley, Foolad and Jones (1993) in tomato and Jeannette et al (2002) reported that growth regressed in beans under the condition of salt stress.
It has been reported that low salt dose in mulberry has a positive effect on plant height growth (Safi, 2012). However, it was stated that the increase in the salt dose shortened the plant height. It was determined that the increase in the salinity of the irrigation water in silage sorghum affected the growth of sorghum negatively and shortened the plant height(Parlak ve Parlak, 2005). Similarly, the best plant height values in Festucaarundinacea were obtained from soils with the lowest salt and Na+ levels. It has been observed that increasing salinity and Na^+ levels cause shortening in plant height (Akıl, 2008). It has also been reported that the increased SAR and salt level in the cane ball cause a shortening of the plant height (Başer, 2015).
In NaCl containing plants, salt stress affects both water uptake and biochemical processes, resulting in reduced plant growth (Parida and Das, 2005). Salt stress causes a decrease in the net photosynthesis rate of plants, adversely affects CO_2 assimilation, greatly reduces nutrient uptake, and finally reduces plant growth and development(Cha-Um and Kirdmanee, 2009).
Germinating seeds do not need to use stored carbohydrates sparingly. Therefore, as the respiration rate increases, the growth rate of the seedlings is expected to be higher. However, if this high respiration rate persists for long periods of time in later growth stages, it can have adverse effects on final product performance. It shows that it uses a high amount of stored carbohydrates for the maintenance of developed organs under conditions of salt stress. This amount of usage also differs according to the varieties(Moud and Maghsoudi 2008).
High NaCl levels negatively affect shoot and root development of plants. Salt stress not only reduces the development and growth of roots (West et al. 2004; Kobayashi et al. 2016; Shelden et al. 2013; Tu et al. 2014), but also prevents dispersal of lateral roots (Julkowska et al. 2014). The properties related to root mass distribution between main and lateral roots (root length and number of lateral roots, etc.) decrease with salt stress (Julkowska et al. 2017). However, the rates of stem (shoot) development and root development affected by salt stress also differ. It is reported that shoot development is more affected by salt stress than root. Huck and Schroeder (1995) and Esechie et al (2002) reported that roots are more tolerant to salt stress than leaves. Germination and development characteristics of grass varieties were investigated in in vitro experiments under salt application. Responses of annual grass varieties to salt stress were different. The cultivars had different characteristics under stress conditions as well as under optimum conditions.
Root length, shoot length, root-shoot fresh weight and root-shoot dry weight were examined in in vitro experiments. Efe and Vallivert cultivars gave the lowest values in the control group in terms of these properties, while Medoacus and Trinova cultivars gave the highest values. In salt application, Tornado, Vallivert, Venus varieties gave the lowest value, while Medoacus and Trinova varieties gave the highest value.

4. CONCLUSION
If grass farming will be carried out on salty soils, their resistance to salt must be determined. However, it is necessary to test the plants at different salt levels in order to decide on the salt dose.

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