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Araştırma Makalesi/*Research Article (Original Paper)*

Determination of Responses of Different Alfalfa (*Medicago sativa* L.) Varieties to Salt Stress at Germination Stage

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Abstract: This study was conducted to determine the responses of some alfalfa varieties to different salt concentrations at germination stage. Alfalfa varieties Bilensoy-80, Alsancak, Gozlu-1, Prosementi and Iside were used as plant materials. Salt concentrations were composed of five levels of NaCl such as 0, 50, 100, 150 and 200 mM. In the experiment, some characters such as germination percentage, plumule length, radicle length, dry weights of plumule and radicle were examined. Results indicated that the salt concentrations above 100 mM had negative effects on germination percentage and dry weights of plumule and radicle. The endurances of varieties to salt stress indicated differences in respect of all components examined. In addition, the effects of variety x salt interactions were significant for lengths of plumule and radicle and plumule dry weight. There were no plumule development in varieties of especially Bilensoy-80, Gozlu-1 and Iside at 200 mM salt level of NaCl. As a brief, the variety Alsancak had been more endurance than the other varieties in experiment.

Key words: Alfalfa, Germination percentage, Salt stress

Farklı Yonca Çeşitlerinin Çimlenme Döneminde Tuz Stresine Tepkilerinin Belirlenmesi

Özet: Bu çalışma, bazı yonca çeşitlerinin çimlenme döneminde farklı tuz konsantrasyonlarına tepkilerinin belirlenmesi amacıyla laboratuvar koşullarında yapılmıştır. Araştırmada bitki materyali olarak Bilensoy, Alsancak, Gözlü, Prosementi ve İside çeşitleri kullanılmıştır. Araştırmada beş farklı NaCl dozu (0, 50, 100, 150 ve 200 mM) ele alınmıştır. Araştırmada çimlenme yüzdesi, sapçık uzunluğu, kökçük uzunluğu, sapçık ve kökçük kuru ağırlıkları gibi özellikler incelenmiştir. Elde edilen sonuçlar çimlenme yüzdesi, sapçık ve kökçük kuru ağırlığında 100 mM NaCl üzerindeki dozlarda olumsuz etkilerin ortaya çıktığını göstermektedir. Çeşitler arasında incelenen tüm özellikler açısından önemli farklılıklar ortaya çıkmıştır. Ayrıca, çeşit x tuz interaksiyonu sapçık ve kökçük uzunluğu ile sapçık kuru ağırlığı açısından önemli çıkmıştır. Araştırmada ele alınan çeşitlerden özellikle Bilensoy-80, Gözlü-1 ve İside çeşitlerinde 200 mM NaCl dozunda sapçık gelişimi olmamıştır. Ele alınan özellikler açısından genel olarak değerlendirildiğinde Alsancak çeşidi çimlenme döneminde tuza dayanım yönünden diğerlerine oranla daha dayanıklı olmuştur.

Anahtar kelimeler: Yonca, Çimlenme yüzdesi ,Tuz stresi

Introduction

Salinity is one of the important environmental factors limiting agricultural production in the world together with the increasing population (Botella et al. 2005). About 10 million hectare land is abandoned due to the salt problem in the world. In the dry and semi-dry climate regions, inadequate rainfall and high transporation are the basic reasons of the saltiness. On the other hand, wrong applications in irrigation especially in the areas of poor drainage may cause saltiness (Baltacı et al. 2004). Determination and use of salt resistant species and varieties are needed to be grown on saline soils where reclamation and practical use are impossible (Karanlık 2001).

Salt accumulation in the soils affect the plant growth differently at different growing stages and the responses of different plants species to salt stress may be different also. The germination and seedling

stages of plants are more sensible to salt stress than the other stages. For this reason, most of the studies are mostly conducted at these stages (Taiz and Zeiger 2002). The basic reason of this negative condition at these stages of plants are the limiting effect of salt on the water absorbtion (Kara et al. 2011). The negative effects of salt stress on seed germination indicate variations depending on plant species (Torech and Thompson 1993). Alfalfa has been characterized as moderataly sensitive to salts with an electrical conductivity (EC) of 2.0 ds/m and a threshold of 1.5 bars osmotic potential of soil solution at field capacity (Maas and Hoffman 1977). In addition, 7 % decrease in alfalfa yields can be expected with each ds/m increase in saturation extract salinity (Rawlins 1979). In contrast, alfalfa has also been characterized as tolerant to salinity with a range of EC values from 6.0 to 8.0 (3840 to 5120 ppm) at which some reduction in growth and yields can be expected (Longenecker and Lyerly 1974). Rizk et al. (1978) in a research conducted with three alfalfa varieties have searched the effects of NaCl and CaCl each applied at 0, 0.01, 0.02 and 0.1 N on germination percentage, germination index, seedling length and dry weight increase. They found that the characters measured have been increasingly more affected as the salt concentrations increased. At the same time, the negative effect of CaCl has been determined less effective than that of NaCl. Al-Saady et al. (2013) reported that the effects of increasing concentrations (4, 6, 8 and 10 ds/m) of salt on germination ratios of alfalfa varieties have been different. In a study conducted by Cacan and Kokten (2014), the effects of the different concentrations (200, 400, 600 and 800 mg/l) of salt on some alfalfa varieties have been tested and the results have indicated that the varieties Basbag, Bilensoy-80, Elci and Kayseri were more tolerant than Savas variety and they displayed negative responses to all increasing salt concentrations for all characters examined in the experiment. In a study conducted to test the tolerance of alfalfa varieties at germination and early seedling stages to osmotic stress caused by salt and PEG 6000 applications Castroluna et al. (2014) examined the germination percentage, leaf and root length, fresh and dry weights of leaves and roots. Researchers had reported that the applications of salt and PEG have reported the germination and there have been differences among all three varieties used in the experiment. This study was conducted to determine the effects of different salt concentrations on germination abilities of some alfalfa varieties grown in Turkey.

Materials and Methods

The study was conducted in the Plant Physiology laboratory of the Department of Field Crops, Faculty of Agriculture, University of Uludag. In the experiment, five varieties of alfalfa such as Bilensoy-80, Alsancak, Gozlu-1, Prosementi and Iside have been tested. Five salt concentrations (0, 50, 100, 150 and 200 mM NaCl) were used as experimental factor. EC values of NaCl levels were 0.0024 ds/m, 5.34 ds/m, 10.33 ds/m, 15.12 ds/m and 19.92 ds/m, respectivelly. The experiment was conducted as Completely Randomized Desing with two factors and four replications. Petri dishes with 15 cm diameter were used for germination. Before germinating, seeds had been subjected to the surface sterilization. For this purpose, sodium hypochloride of 5 % has been used. Seeds have been shaken for 5 min with sodium hypochloride and then have been washed well (Babakhani et al. 2011). Surface strerilized seeds have been taken on drying papers and dried and after this 50 seeds were placed among each petri dish icluding double layered filter papers. After this procedure, 15 ml of each different salt solution was pured over each petri dish to treat seeds. Petri dishes were wrapped with parafilm to prevent evaporation. Immediately after these processes were put into a dark chamber and adjusted to a temperature of 20±1ºC and kept here for 10 days (Sehirali 1997; Castroluna et al. 2014).

In the experiment, observations have been taken every day at same time and the seeds passing beyond 2 mm were accepted as germinated ones (Soltani et al. 2012). At the end of 10 days, all of the germinated seeds were counted and the germination percentage (%) was calculated (Scott et al. 1984). At the 10 th day of germination, 10 seedling were taken from each petri dish and the lengths of plumule and radicle were measured on these samples. In addition, samples were sorted into radicle and plumule and they were dried at 70 ºC for 6 hours and then were weighed (Al-Saady et al. 2013).

The data of this experiment were subjected to variance analysis in accordance with Completely Randomized desing (Turan 1995). All of the calculations were made in MINITAB and MSTAT-C programs. The possibility levels of 1 % and 5 % were used for significant tests, and 5 % level for determination of groups. LSD test was used for determination of different groups.

Results and Discussion

The results of variance analysis related to germination percentages, lengths of plumule and radicle, dry weights of plumule and radicle of some alfalfa varieties exposed to different concentrations of salt concentration were shown at Table 1. The varieties indicated differences in respect of radicle dry weight at 5 % possibility level, germination percentage, lengths of plumule and radicle and plumule dry weight at 1 % possibility level. All of the salt concentrations had affected all of the characters examined. The effects of variety x salt concentration interaction have been found statistically significant on the plumule length, radicle length and plumule dry weight at 1 % possibility level (Table 1). The effects of variety x salt concentration interaction to be found significant may be due to the different responses of alfalfa varieties to NaCl.

Table 1. Results of analysis of variance in determined traits (Mean squares)

Variance Source	df	Germination Percentage	Plumule Length	Radicle Length	Plumule Dry Weight	Radicle Dry Weight
Variety (V)	4	217.74**	5.1247**	5.2215**	8.238**	1.7657*
Salt (S)	4	431.54**	37.4324**	13.9380**	95.443**	11.1901**
V x S	16	28.19	0.6444**	0.7047**	3.318**	0.6467
Error	75	21.12	0.2240	0.2049	0.351	0.5471

df: degrees of freedom, *P<0.05 and **P<0.01.

Germination percentage (%)

Germination percentages of varieties indicated significant differences and the highest ratios were produced by Bilensoy-80, Alsancak and Gozlu-1 (Table 2). On the other hand, the effects of salt concentrations on germination percentages of varieties were of significance and the germination percentages progressively decreased depending on the increasing salt concentrations. Thus, the highest germination percentage (96.08 %) was obtained from control (0 mM NaCl) and the lowest germination percentage (85.30 %) from the highest salt concentration (Table 2). This kind of results is to arise from negative effetcs of water absorbtion and toxic effects caused by salt ions (Ekmekci et al., 2005). The responses of varieties to salt concentrations were similar and from this reason the effects of variety x salt concentration interaction were insignificant (Table 1 and Table 2). The similar results in regard to the negative effects of salt concentrations on germination percentages of varieties were reported by other researchers (Rizk et al. 1978; Al-Saady et al.2013; Bhardwaj et al. 2010; Hamidi and Safarnejad 2010; Torabi et al. 2011; Soltani et al. 2012; Castroluna et al. 2014).

Table 2. Average value of germination percentage (%) of some alfalfa varieties grown in different salt concentrations

	Salt Concentration (mM)					
Variety	0	50	100	150	200	Mean
Bilensoy-80	97.50	96.00	94.50	93.50	94.00	95.10[a]
Alsancak	99.50	97.00	95.00	91.00	88.50	94.20[a]
Gozlu-1	99.00	97.00	93.00	89.00	85.50	92.70[a]
Prosementi	96.50	94.50	91.00	88.00	76.00	89.20[b]
Iside	91.50	91.00	88.50	83.50	82.50	87.40[b]
Mean	96.80[a]	95.10[ab]	92.40[b]	89.00[c]	85.30[d]	

Means of the variety and salt concentration followed by the same letter were not significantly different at the 0.05 level using LSD test

Plumule length (cm)

The longest plumule length was observed from Alsancak variety following by Bilensoy-80 and Gozlu-1 varieties. Mean of plumule lenght varied between 0.28 and 3.81 cm for salt concentrations. The longest plumule lenght (3.81 cm) was observed from control. Generally plumule lenght decreased as salt concentration increased (Table 3).

The effects of variety x salt concentration interaction have been found statistically significant on the plumule length (Table 1). The plumule lengths of alfalfa varieties exposed to different salt concentrations varied between 0.00-4.42 cm. The longest plumule length was determined at Alsancak and Gozlu-1 varieties untreated with salt and at Alsancak variety treated with 50 mM NaCl. On the other hand, the shortest plumule lengths were determined at 200 mM NaCl level in Bilensoy-80, Gozlu-1 and Iside variety as 0.00 cm. In general, while salt concentrations increased the plumule lengths decreased. However, the responses of varieties to salt concentrations showed differences. This condition is to result from interaction effects of variety x salt concentration. For example, Alsancak was more resistant to salt stress at 50 mM NaCl than the other varieties. Bilensoy-80, Gozlu-1 and Iside were more sensitive to salt concentrations than the other varieties (Table 3). That the increasing salt concentrations have affected the plumule lengths at varying rates was reported by many other researchers (Avcıoglu et al. 2003; Bhardwaj et al. 2010; Soltani et al. 2012; Cacan and Kokten 2014).

Radicle length (cm)

Among various varieties the highest radicle lenght was observed from Alsancak variety with 2.67 cm following by Bilensoy-80 (2.02 cm) and Gozlu-1 (2.10 cm) varieties. Salt concentrations means showed that maximum radicle lenght was recorded under control (0 mM NaCl). Generally radicle lenght decreased as salt concentration increased (Table 3).

Table 3. Average value of plumule length (cm) and radicle length (cm) of some alfalfa varieties grown in different salt concentrations

Variety	Salt Concentration (mM)					Mean
	0	50	100	150	200	
	Plumule Length (cm)					
Bilensoy-80	3.91^{ab}	3.50^{bc}	1.94^{gh}	1.85^{gh}	0.00^{k}	2.24^{b}
Alsancak	4.39^{a}	4.39^{a}	2.34^{fg}	$1.76^{g-ı}$	1.05^{j}	2.78a
Gozlu-1	4.42^{a}	3.01^{c-e}	2.71^{ef}	$1.74^{g-ı}$	0.00^{k}	2.38^{b}
Prosementi	3.41^{b-d}	2.78^{d-f}	1.71^{g-j}	1.36^{h-j}	0.36^{k}	1.92^{c}
Iside	2.93^{c-f}	$1.77^{g-ı}$	1.32^{h-j}	1.15^{ij}	0.00^{k}	1.43^{d}
Mean	3.81^{a}	3.09^{b}	2.00^{c}	1.57^{d}	0.28^{e}	
	Radicle Length (cm)					
Bilensoy-80	3.01^{bc}	2.68^{cd}	1.89^{e-h}	$1.58^{\text{ I-j}}$	$0.94^{\text{ kl}}$	2.02^{b}
Alsancak	3.91^{a}	3.91^{a}	2.93^{bc}	$1.62^{e-ı}$	$0.95^{\text{ j-l}}$	2.67^{a}
Gozlu-1	3.49^{ab}	$1.99^{\text{ e-g}}$	2.68^{cd}	$1.47^{\text{ g-k}}$	$0.89^{\text{ kl}}$	2.10^{b}
Prosementi	2.25^{de}	$1.68^{e-ı}$	1.48^{g-k}	1.34^{h-k}	0.64^{l}	1.48^{c}
Iside	2.16^{d-f}	$1.66^{e-ı}$	1.45^{g-k}	$1.20^{ı-l}$	0.64^{l}	1.42^{c}
Mean	2.96^{a}	2.38^{b}	2.08^{c}	1.44^{d}	0.81^{e}	

Means of the variety, salt concentration and variety x salt concentration interactions followed by the same letter were not significantly different at the 0.05 level using LSD test

The effects of variety x salt concentration interaction have been found statistically significant on the radicle length (Table 1). The radicle lengths of alfalfa varietis were negatively affected by different salt concentrations and the values of radicle lengths varied between 0.64 and 3.91 cm. The longest radicle length (3.91 cm) was determined in Alsancak variety at control (0 mM NaCl) and 50 mM NaCl, the shortest radicle length (0.64 cm) in Prosementi and Iside varieties at 200 mM NaCl (Table 3). The response of radice length of Alsancak to salt stress was similar to the response of its plumule length to salt stress. That is plumule length of Alsancak at 50 mM NaCl was not affected and its value was similar to that of control. This case may be arosen from the lack of toxic effect of 50 mM NaCl both on plumule and radicle lengths. As a matter of fact, Castroluna et al. (2014) determined that alfalfa varieties indicated important differences aganist to NaCl concentrations in respect of their plumule and radicle lengths, and that the lengths of plumule nd radicle decreased in some varieites as concentrations of salt increased, and even obtained deadly results while determined no negative even obtained positive effects of salt concentrations on plumule and radicle lengths in some varieties. In our research, the important decrease in radicle length started from 100 mM NaCl in all varieties but Gozlu-1. In Gozlu-1, the radicle length decreased 45 % at 50 mM NaCl compared to control but this effect disappeared at and became stable from 100 mM NaCl. Castroluna et al. (2014) reported that increasing salt concentrations until certain point increased radicle length and decreased it at 100 mM and then increased again at 200 mM NaCl in Salina variety of alfalfa. Primary root system of alfalfa exposed directly to NaCl treatment prevented the

root growth by depressing the cell enlargement and cycle of cell (Wang et al. 2009). At the same time, the root hairs lose their activities and then disappear depending an increasig levels of NaCl (Ali et al. 1999).

Plumule dry weight (mg seedling^{-1})

The plumule dry weights of alfalfa varieties showed significant differences and the highest dry weight (4.53 mg seedling^{-1}) was determined in Gozlu-1 following by Alsancak. Effects of salt concentrations on plumule dry weights of varieties were also found significant. In general, the highest plumule dry weights have been determined at control and 50 mM NaCl, while the lowest values were obtained at 200 mM NaCl (Table 4).

The effects of variety x salt concentration interaction have been found statistically significant on the plumule dry weight (Table 1). Dry weights of varieties of alfalfa have been affected by salt concentrations their values varied between 0.00 and 1.04 mg seedling^{-1}. The highest value of plumule dry weight was determined in Gozlu-1 variety as 7.49 and 7.22 mg seedling^{-1} at control and 50 mM NaCl and these values were followed by 6.87 mg seedling^{-1} in Alsancak variety at 50 mM NaCl. Whereas, the lowest dry weight of plumule was produced in Bilensoy-80, Gozlu-1 and Iside varieties at 200 mM NaCl. Although the variety Gozlu-1 resisted in the begining to the increasing salt concentrations up to certain point it did not keep this stability at the highest concentration of NaCl (Table 4). That the increasing salt concentrations did not caused important decreases in dry weight of plumule of some alfalfa varieties at first concentrations of salt was reported by Cacan and Kökten (2014). Whereas, Castroluna et al. (2014) reported that the dry weight of plumule indicated increases through the increasing salt concentrations. In the present research, Bilensoy-80, Gozlu-1 and Iside have been mostly affected varieties from salt stress when plumule dry weight was considered. Although the responses of these varieties to salt stress had not been too higher at low concentrations, they did not survived at 200 mM NaCl.

Table 4. Average value of plumule dry weight (mg seedling^{-1}) and radicle dry weight (mg seedling^{-1}) of some alfalfa varieties grown in different salt concentrations

Variety	Salt Concentration (mM)					Mean
	0	50	100	150	200	
	Plumule Dry Weight (mg seedling^{-1})					
Bilensoy-80	6.09^{b-d}	5.31^{d-f}	4.95^{e-g}	3.21^{ij}	0.00^{n}	3.91^{bc}
Alsancak	6.21^{bc}	6.87^{ab}	$2.78^{ı-k}$	$3.01^{ı-k}$	1.94^{l}	4.16^{ab}
Gozlu-1	7.49^{a}	7.22^{a}	4.64^{fg}	3.30^{ij}	0.00^{n}	4.53^{a}
Prosementi	4.69^{fg}	$3.58^{hı}$	2.48^{j-l}	2.35^{kl}	1.04^{m}	2.83^{d}
Iside	5.54^{c-e}	5.17^{ef}	4.32^{gh}	$3.15^{ı-k}$	0.00^{n}	3.63^{c}
Mean	6.00^{a}	5.63^{a}	3.83^{b}	3.00^{c}	0.60^{d}	
	Radicle Dry Weight (mg seedling^{-1})					
Bilensoy-80	3.47	2.54	2.70	1.68	1.14	2.31^{ab}
Alsancak	3.24	2.78	1.95	1.54	1.16	2.13^{b}
Gozlu-1	3.96	3.89	2.73	1.67	1.60	2.77^{a}
Prosementi	2.34	2.58	2.18	2.16	1.88	2.23^{b}
Iside	2.95	2.21	2.12	1.52	1.11	1.98^{b}
Mean	3.19^{a}	2.80^{a}	2.33^{b}	1.71^{c}	1.38^{c}	

Means of the variety, salt concentration and variety x salt concentration interactions followed by the same letter were not significantly different at the 0.05 level using LSD test

Radicle dry weight (mg seedling^{-1})

The radicle dry weights of alfalfa varieties showed significant differences and the highest dry weight (2.77 mg seedling^{-1}) was determined in Gozlu-1, following by Bilensoy-80 (2.31 mg seedling^{-1}). On the other hand, effects of salt concentrations on radicle dry weights of varieties were also found significant. In general, the highest radicle dry weights have been determined at control and 50 mM NaCl, while the lowest values were obtained at 150 and 200 mM NaCl (Table 4). Radicle dry weights have decreased between 50 and 150 mM NaCl and then the decrease was not found significant. It seems that the salt ions at low salt concentrations did not make toxic effect on root growth. Castroluna et al (2014) reported that the salt level of 50 mM NaCl encoraged root growth, but that there were no differences among concentrations. In contrast of this, Cacan and Kokten (2014) determined that there were differences

among varieties aganist different salt concentrations and that in general, depending on increasing salt concentrations the radicle dry weights decreased significantly. In our study, dry weights of radicle in varieties responded similarly to salt concentrations and were found no differences among their responses when compared. As a general rule, increasing rates of NaCl decreased progressively the radicle dry weights. Therefore, the effects of variety x salt conentration interaction were found insignificant (Table 1 and Table 2). In the experiment, the effects of salt concentrations on radicle growth were less than on plumule growth. The severity of salt stress on plumule growth was so great that the growth expired at 200 mM NaCl. Although the root system is expoesd directly salt stress, the upper ground parts of plants are more sensitive to salt, and for this reason the root/shoot ratio in plants increases under salt stress conditions. Although the mechanism of this event is not explained yet, the different changes determined in cell walls of root and shoot are indicated as a reason for this incident (Munns and Tester 2008).

Conclusion

In this study, the effects of different salt concentrations on some alfalfa varieties have been searched and found that all of the characters examined have been negatively and significantly affected. In addition, the effects of variety x salt concentration interactions were of significance and this interaction effect indicated that the responses of different varieties to salt stress were also different. That is, the negative effects of salt concentrations on some varieties were higher than on the others. In some varieties the negative effects began after 100 mM NaCl and reached peak level at 200 mM NaCl causing a complete growth expiration. In a general evaluation of results, Alsancak variety showed more expectation than the others in respect of salt resistance during germination stage.

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