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Effects of Prolonged High Salinity Stress on Alfalfa (*Medicago sativa* L.) Cultivars and Populations

Berna Efe[1], Namuk Ergün[2], Cengiz Sancak[3]

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ABSTRACT

Background: Alfalfa is a promising crop for improving soil structure and restoring agricultural land, particularly in areas affected by salinity. However, high and continuing salinity in soil and irrigation water can pose a significant challenge to the growth and productivity of alfalfa. The objective of this study was to investigate the effects of prolonged high salinity stress on alfalfa cultivars and populations.

Methods: The material used in this study included 16 alfalfa populations and four alfalfa cultivars that exhibit salt tolerance during germination and early seedling development. The materials were exposed to 17.52 dS m^{-1} saline irrigation water stress for three months. As well as the morphology, survival at three months and crude ash, sodium and chloride ions were measured.

Result: Salt-tolerant plants showed a slowdown in growth compared to control conditions, although they were still able to maintain growth. Duration and severity of stress affected the salt tolerance of the genotypes. The survival rates of the materials remained relatively unchanged for the first two months, but decreased significantly by the end of the third month. Furthermore, the survival rate of the materials increased as the amount of sodium and chloride ions absorbed by the plant decreased under high salinity conditions. Genetic material demonstrating long-term high salinity tolerance is crucial for resistance breeding and holds promise for the development of tolerant cultivars that can be grown under field conditions in the future. The properties of this material need to be determined and its long-term performance in field conditions needs to be tested.

Key words: Crude ash, High salt stress, Morphological traits, Na and Cl ions, Surviving plants.

INTRODUCTION

Stress factors such as extreme temperatures, waterlogging or drought, high salinity, heavy metals and ultraviolet radiation affect growth and development, leading to large yield losses (He *et al*., 2018). Salinity is one of the stresses that cause a decrease in agricultural yield in arid and semi-arid regions. Today, one-fifth of the irrigated agricultural land is affected by salinity, while about 800 million hectares of land worldwide are under the influence of this stress. It is recognized as a global threat, with estimates suggesting that around 50% of cultivated land will face this stress in the future (Khan *et al*., 2022; Li *et al*., 2022).

Plants can adapt to survive under stressful conditions (Song *et al*., 2019). Salt tolerance is controlled by a large number of genes, which means that plants have different resistance strategies to this stress (Yılmaz *et al*., 2011). While some plants are sensitive to saline conditions, others show tolerance with different mechanisms. These, tolerance mechanisms can be physiological, biochemical and molecular (Culha and Cakırlar, 2011). Although several candidate genes have been identified and used in plants to develop different mechanisms to adapt to salt stress, further progress is required for the successful use of salt-tolerant plants under field conditions (Muchate *et al*., 2016).

The effects of salt stress vary depending on the type of salt, the level and duration of the stress, the genetic structure and developmental stage of the stressed plants (Zhu, 2002; Culha and Cakırlar, 2011). Minimizing the impact of stress and understanding the genetic differences that contribute to different levels of stress tolerance is crucial. Genetic diversity plays a significant role in helping some species adapt to changes in their environment (Jabri *et al*., 2021).

Alfalfa is a perennial forage crop that exhibits moderate salt tolerance and demonstrates diversity both within and between populations (Soltani *et al*., 2012; Bhattarai *et al*., 2020). Its wide genetic diversity may make it a good candidate for the development of salt-tolerant varieties and for the assessment and remediation of salt-affected areas (Soufan *et al*., 2022).

The salt tolerance strategy of alfalfa is a complex process and it has been determined as having different

[1]Department of Meadow-Rangeland and Forage Crops, Central Research Institute for Field Crops, Ankara, Türkiye.
[2]Department of Breeding and Genetics, Central Research Institute for Field Crops, Ankara, Türkiye.
[3]Department of Field Crops, Faculty of Agriculture, Ankara University, Ankara, Türkiye.

Corresponding Author: Berna Efe, Department of Meadow-Rangeland and Forage Crops, Central Research Institute for Field Crops, Ankara, Türkiye. Email: bernaefe85@gmail.com

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mechanisms for coping with high salinity (Madhava Rao *et al*., 2006; Anower *et al*., 2013). Morphological, physiological and biochemical responses of alfalfa crops to stress during germination and seedling development periods have been investigated in many studies (Soltani *et al*., 2012; Valizadeh *et al*., 2013; Tani *et al*., 2018; Hou *et al*., 2022). However, when developing tolerant varieties for field conditions, it is necessary to evaluate the ability of alfalfa plants to adapt to long-term high salt stress. This involves evaluating the various mechanisms that plants employ to deal with salt stress.

According to Tarchoune *et al*. (2012), NaCl is the primary compound responsible for causing salt stress in plants. It was reported that the accumulation of Na^+ and Cl^- ions was lower in tolerant alfalfa genotypes under saline conditions and positive correlations with salt tolerance index, height and number of shoots were observed for shoot biomass yield at high salinity (Sandhu *et al*., 2017).

In this research three components were evaluated namely i) the time-dependent effect of extreme salinity on 16 alfalfa populations and 4 alfalfa cultivars tolerant to NaCl salt stress, ii) survival rate among this material and iii) possibilities for use in breeding tolerant cultivars.

MATERIALS AND METHODS

Alfalfa cultivars (Defne, Alsancak, Özpınar, Bilensoy-80) and populations (L-1740, L-1741, L-1743, L-1744, L-1754, L-1756, L-1757, L-1758, L-1759, L-1761, L-1763, L-1771, L-1872, L-1820, L-1867, L-2209) which previously demonstrated tolerance to 17.52 dS m^{-1} salinity of irrigation water were evaluated as plant material in this study.

The experiments were conducted under controlled greenhouse conditions (20°C±2°C) in a randomized block design with four replications in 2021 growing season at the Central Research Institute for Field Crops. Alfalfa cultivars and populations were treated with two contrasting irrigation water salinities for three time periods (30, 60 and 90 days). In the control treatment, plants were irrigated with water with an electrical conductivity (EC) of 0.60 dS m^{-1}, representing low salinity conditions. For the salt stress treatment, plants were irrigated with water having an EC of 17.52 dS m^{-1}, effectively inducing high salinity stress by sodium chloride (NaCl). In both the control and salt stress treatments, irrigation was applied three times a week with a leaching rate of 20%. Electrical conductivity of the peat in pots before both irrigation treatments was 3.12 dS m^{-1}. After 30, 60 and 90 days, the electrical conductivity of the used peat in the control group was 4.15, 4.06 and 7.57 dS m^{-1}, while in the stress group it was 19.41, 31.35 and 62.70 dS m^{-1}.

In both treatments, root and shoot length, number of leaves, leaf width and length, leaf, stem and root fresh and dry weights were determined by measuring, weighing and counting at 30, 60 and 90 days. Dry weights were obtained by weighing after drying at 70°C for 48 hours (Turk *et al*., 2018). Furthermore, the amount of crude ash, sodium (Na^+) and chloride (Cl^-) ions within the plant body of the populations and varieties were detected. Crude ash was determined by the Ash - Basic method (AACC method 08-01.01), Sodium - Ammon. Ox-ICP, Chlorine - Mohr titrimetric methods.

The statistical significance levels of differences between genotypes, treatments, times and their interactions for each character in the experiment were determined using combined analysis of variance (ANOVA). In addition, grouping of means was performed by Student's t multiple comparison tests (LSD) (Montgomery, 2013). Standard deviation (SD) and coefficient of variation (CV) were calculated for each traits to determine the distribution of the characters around the mean. Moreover, Pearson's Correlation analysis was performed according to Crawford (2006) on the morphological and agronomic characters examined in the material to determine the relationships between traits under salt stress. Time-dependent distribution was virtualized by plotting xy distribution graphs according to measurements at 30, 60 and 90 days for the changes in traits in alfalfa genotypes.

RESULTS AND DISCUSSION

The study evaluated the effects of high salinity (17.52 dS m^{-1} irrigation water) on alfalfa cultivars/populations over time. Table 1 presents the results of analysis of variance conducted on eleven different characteristics across 16 alfalfa populations and 4 alfalfa cultivars, both under control and salt stress conditions. Significant differences were found in the examined traits at the $p<0.01$ level, including genotypes, treatments, time, genotype × treatment, genotype × time, treatment × time and genotype × treatment × time interactions.

Large variations were observed in most of the traits studied in the alfalfa genotypes. To determine these variations, the standard deviation and coefficient of variation ratio were calculated for each trait. Table 2 displays the average, minimum, maximum, standard deviation and coefficient of variation values for the traits investigated. The table shows that the coefficient of variation values generally increased under salt stress for all traits except for the number of leaves (NOL). High salinity particularly increased the variation in leaf, stem and root weights of alfalfa cultivars/populations. All traits exhibited a decrease in plant growth during the three-month period under high salt stress. Over a period of three months, shoot length (SL) decreased by 78.15%, 81.56% and 80.85% in comparison to the control group, while root length (RL) declined by 66.89% and 73.76% in the first two and third months, respectively. Additionally, under stress, the number of leaves (NOL) reduced by 54.68% in the first month, by 72.81% in the second month and by 83.03% in the third month. The rate of decrease in leaf width (LW) and leaf length (LL) also decreased over the three-month period. Compared to the control under stress, the reductions in LW were 45.98%, 36.69% and 21.21%, while in LL they were 42.19%, 32.39%

Table 1: Analysis of variance on eleven characters in 16 alfalfa populations and 4 alfalfa cultivars under control and salt stress conditions.

Character		Genotype	Treatment	Time	Genotype × Treatment	Genotype × Time	Treatment × Time	Genotype × Treatment × Time	Error	CV (%)
DF		19	1	2	19	19	19	19	360	
SL (cm)	MS	103.82	64817.91	5032.10	89.72	42.30	2590.96	39.51	15.03	22.47
	F	6.91**	4311.76**	334.74**	5.97**	2.81**	172.35**	2.63**	-	
RL (cm)	MS	47.59	27942.08	1447.56	45.81	59.82	84.74	42.63	22.92	29.53
	F	2.08**	1219.32**	63.17**	2.00**	2.61**	3.70*	1.86**	-	
NOL (pieces)	MS	201.58	41416.06	14303.10	188.53	71.54	9706.22	63.92	30.31	36.87
	F	6.65**	1366.34**	471.87**	6.22**	2.36**	320.21**	2.11**	-	
LW (mm)	MS	9.61	1550.92	1.28	4.14	6.16	95.48	3.61	1.46	14.44
	F	6.56**	1059.33**	0.88**	2.83**	4.21**	65.21**	2.46**	-	
LL (mm)	MS	8.89	730.17	11.18	2.63	4.24	50.93	2.18	0.85	13.57
	F	10.41**	854.45**	13.08**	3.07**	4.96**	59.60**	2.55**	-	
LFW (mg)	MS	156050.44	41007741.39	9273306.25	150361.45	56722.93	8163822.62	45732.07	21234.19	39.56
	F	7.35**	1931.21**	436.72**	7.08**	2.67**	384.47**	2.15**	-	
LDW (mg)	MS	7645.45	1167341.94	351100.27	6812.54	2341.00	215413.22	1953.49	775.75	52.87
	F	9.86**	1504.79**	452.59**	8.78**	3.02**	277.68**	2.52**	-	
SFW (mg)	MS	145895.83	35139683.50	9087283.50	148602.61	66313.60	8186403.10	63412.09	27897.33	45.22
	F	5.23**	1259.61**	325.74**	5.33**	2.38**	293.45**	2.27**	-	
SDW (mg)	MS	11222.58	2205719.39	838512.56	11182.87	4847.64	622828.99	4819.04	1789.70	43.42
	F	6.27**	1232.45**	468.52**	6.25**	2.71**	348.01**	2.69**	-	
RFW (mg)	MS	135409.25	38429118.34	17065325.31	133291.45	100621.33	14439698.81	92955.45	23111.63	52.94
	F	5.86**	1662.76**	738.39**	5.77**	4.35**	624.78**	4.02**	-	
RDW (mg)	MS	19882.78	3571210.01	1989705.32	19185.11	13792.19	1523005.23	13002.08	2360.78	48.98
	F	8.42**	1512.73**	842.82**	8.13**	5.84**	645.13**	5.51**	-	

**: Statistically significant at 0.01 probability level, DF: Degrees of freedom, SL: Shoot length (cm); RL: Root length (cm); NOL: Number of leaves (pieces); LW: Leaf width (mm); LL: Leaf length (mm); LFW: Leaf fresh weight (mg); LDW: Leaf dry weight (mg); SFW: Stem fresh weight (mg); SDW: Stem dry weight (mg); RFW: Root fresh weight (mg); RDW: Root dry weight (mg); MS: Mean square; F: F value; CV: Coefficient of variation (%).

Table 2: The average, minimum, maximum, standard deviation and coefficient of variation values of the analyzed characters.

Traits	Treatment	Mean±Sdtdev	Change (%)	Min-Max	CV (%)
SL (cm)	Control-30th days	19.28±4.58	-78.15	11.68-26.23	23.74
	Salt-30th days	4.21±1.25		2.25-7.70	29.71
	Control-60th days	28.81±6.36	-81.56	18.9-41.68	22.09
	Salt-60th days	5.31±1.48		2.90-8.60	27.81
	Control-90th days	38.54±4.38	-80.85	29.78-46.60	11.35
	Salt-90th days	7.38±2.23		3.15-11.90	30.19
RL (cm)	Control-30th days	21.26±3.93	-66.89	15.73-32.23	18.47
	Salt-30th days	7.04±1.47		4.28-9.95	20.84
	Control-60th days	21.26±3.94	-66.89	16.58-29.53	17.83
	Salt-60th days	7.04±2.32		3.63-14.45	30.97
	Control-90th days	28.12±5.15	-73.76	20.13-42.50	18.33
	Salt-90th days	7.38±3.08		6.30-17.05	27.48
NOL (pieces)	Control-30th days	8.41±1.71	-54.68	5.75-11.25	20.28
	Salt-30th days	3.81±0.66		2.75-5.75	17.40
	Control-60th days	21.65±6.36	-72.81	8.50-30.50	29.37
	Salt-60th days	5.89±1.24		4.00-8.25	21.14
	Control-90th days	42.60±10.88	-83.03	22.50-64.25	25.54
	Salt-90th days	7.23±1.25		5.00-9.50	17.35
LW (mm)	Control-30th days	10.95±0.65	-45.98	9.65-12.01	5.92
	Salt-30th days	5.92±0.82		4.12-7.61	13.86
	Control-60th days	10.32±1.69	-36.69	7.34-14.49	16.43
	Salt-60th days	6.53±1.07		4.48-9.04	16.38
	Control-90th days	9.26±1.43	-21.21	6.69-12.58	15.47
	Salt-90th days	7.29±1.08		5.57-9.71	14.78
LL (mm)	Control-30th days	8.27±0.60	-42.19	7.39-9.89	7.30
	Salt-30th days	4.78±0.57		3.30-5.74	12.01
	Control-60th days	8.20±1.32	-32.39	6.11-11.98	16.14
	Salt-60th days	5.54±1.05		3.65-8.22	18.94
	Control-90th days	7.67±1.19	-16.37	5.45-10.13	15.56
	Salt-90th days	6.42±1.06		4.62-9.03	16.48
LFW (mg)	Control-30th days	262.00±93.96	-78.74	122.25-421.95	35.86
	Salt-30th days	55.71±20.59		30.88-96.43	36.95
	Control-60th days	546.09±219.37	-84.72	169.45-965.23	40.17
	Salt-60th days	83.44±34.01		26.80-149.08	40.76
	Control-90th days	1173.73±259.28	-92.42	600.70-1523.30	22.09
	Salt-90th days	88.93±45.56		25.47-221.55	51.24
LDW (mg)	Control-30th days	31.47±10.40	-79.26	16.53-48.90	33.06
	Salt-30th days	6.53±2.19		3.45-11.55	33.49
	Control-60th days	110.55±43.55	-89.77	27.55-175.15	39.40
	Salt-60th days	11.31±7.08		3.20-35.15	62.65
	Control-90th days	198.37±59.99	-86.56	84.65-295.53	30.24
	Salt-90th days	26.66±10.12		11.75-50.00	37.97
SFW (mg)	Control-30th days	193.59±80.45	-85.10	75.33-335.10	41.55
	Salt-30th days	28.85±14.86		10.95-57.38	51.51
	Control-60th days	466.78±194.33	-89.04	135.35-832.30	41.63
	Salt-60th days	51.14±19.49		21.93-84.50	38.11
	Control-90th days	1099.06±305.22	-94.90	548.05-1601.90	27.77
	Salt-90th days	56.02±22.11		13.35-100.85	39.47

Table 2: Continue...

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SDW (mg)	Control-30th days	28.70±12.84	-88.17	11.40-50.93	44.74
	Salt-30th days	3.40±1.20		1.88-5.90	35.21
	Control-60th days	120.34±50.83	-91.74	31.90-192.53	42.23
	Salt-60th days	9.95±5.42		2.50-23.50	54.51
	Control-90th days	294.07±87.03	-92.16	128.63-453.85	29.59
	Salt-90th days	23.04±9.08		6.00-39.25	39.39
RFW (mg)	Control-30th days	144.68±54.65	-76.37	73.43-314.33	37.77
	Salt-30th days	34.19±16.70		11.80-69.88	48.85
	Control-60th days	382.72±146.97	-88.93	98.43-643.08	38.40
	Salt-60th days	42.37±27.61		9.35-123.13	65.17
	Control-90th days	1329.95±368.80	-93.75	692.95-1996.50	27.73
	Salt-90th days	83.08±48.15		4.80-201.75	57.95
RDW (mg)	Control-30th days	15.85±6.22	-80.48	8.45-33.68	39.23
	Salt-30th days	3.10±1.11		1.48-5.65	35.86
	Control-60th days	121.45±55.36	-94.51	28.40-272.38	45.59
	Salt-60th days	6.66±6.57		0.95-30.03	98.62
	Control-90th days	419.04±140.76	-93.07	163.90-676.65	33.59
	Salt-90th days	29.05±14.27		6.75-55.55	49.13

and 16.37% in that order. The reduction in LFW (leaf fresh weight) was 78.74%, 84.72% and 92.42% in the first, second and third month, respectively. A significant reduction in plant biomass was observed across all measured parameters. Stem fresh weight (SFW) exhibited a marked decrease of 85.10%, 89.04% and 94.90% in the first, second and third months, respectively. Root fresh weight (RFW) followed a similar trend, declining by 76.37%, 88.93% and 93.75% in that order. Leaf dry weight (LDW) displayed a comparable reduction of 79.26%, 89.77% and 86.56% across the three months. Stem dry weight (SDW) demonstrated a consistent decrease of 88.17%, 91.74% and 92.16%, respectively. Finally, root dry weight (RDW) exhibited a significant decline of 80.48%, 94.51% and 93.07%, respectively.

Fig 1 shows the results of the correlation analysis performed to determine the degree of relationship between the traits under stress. The analysis revealed a strong correlation (0.92) between SFW and RFW, with LFW also showing a high correlation with these traits (0.82 and 0.76, respectively). The correlation between LW and LL as well as SL and SDW is quite high and in the same ratio (0.90). The correlation coefficient between NOL and LW characters was found to be -0.32, indicating a weak negative relationship. This was followed by the similarity between NOL and LL traits, which was -0.17.

Table 3 shows the amount of crude ash, sodium and chloride ions in the plants at the end of the 90 days of stress. The stressed plants had lower crude ash ratios compared to the control, except for the L-1744, L-1763, L-1771, L-1758 and L-1867 populations. Additionally, all salt-stressed materials showed a significant increase in sodium and chloride ions. The crude ash content was highest in the control group for the Özpınar cultivar at 23.49% and under stress, it was highest for the L-1758 population at 18.48%. Among the genotypes, the lowest crude ash content in the control group was L-1758 with 13.47%, while the lowest crude ash content in the stress group was L-1741 with 14.02%. In the control plants, the Na ion content ranged from 0.09% (Bilensoy-80) to 0.25% (L-1744). Under stress, L-2209 had the lowest Na content (3.36%), while L-1763 had the highest (6.87%). In the control group, the lowest and highest amounts of Cl ions were 1.38% (Bilensoy-80) and 2.05% (L-1872), respectively. In the salt stress group, these amounts ranged from 4.68% (L-1820) to 11.50% (L-1763).

When the survival rate of the material under high salt stress was evaluated over a three month period, it was relatively unchanged for the first two months (Fig 2). By the end of the third month, there is a significant reduction in the number of surviving individuals. At the end of the first and second months, the population with the highest number of individuals surviving at high salinity was L-1740 (83.33% and 80.56%, respectively), while the population with the lowest number of survivors was L-1754 (33.33%). At the end of the third month, the Defne cultivar had the highest survival rate under stress at 50.00%, while the L-1741, L-1758 and L-1867 populations had the lowest at 19.44%.

Fig 3 displays the sodium, chloride content and survival rates of the plants at the end of three months of salt stress. The figure displays the survival rates in relation to the sodium (Na^+) ion content on the y-axis and the chloride (Cl^-) ion content on the x-axis. The colored legend indicates the corresponding survival rates. In the legend, the color yellow shows a high survival rate, while purple indicates a low survival rate at the end of the ninety days. When Na, Cl contents and survival rates of the genotypes at the end of the ninety days are evaluated together, it is observed that Na and Cl accumulation is generally lower in genotypes with high survival rates. The cultivar Defne, highlighted in light yellow, has the highest survival rate among the

materials and notably low sodium content in the plant. However, high levels of Na and Cl were found in L-1867, a population with a low survival rate (shown in dark purple color). Nevertheless, it is possible to observe genotypes that do not conform to this relationship. For instance, despite having low Na and Cl contents, the survival rates of cultivar Bilensoy-80, populations L-1756 and L-1820 were not as high as expected (Fig 3).

The growth of alfalfa plants is influenced both by the genetic variability of the plants and by the environmental conditions in which they are grown. Environmental stresses such as salinity and drought are the main factors limiting plant growth and yield in alfalfa. Leaf size in alfalfa varies considerably according to climatic parameters and environmental factors such as disease-pest and stress factors. Nan *et al*. (2019) reported that alfalfa leaf size varies

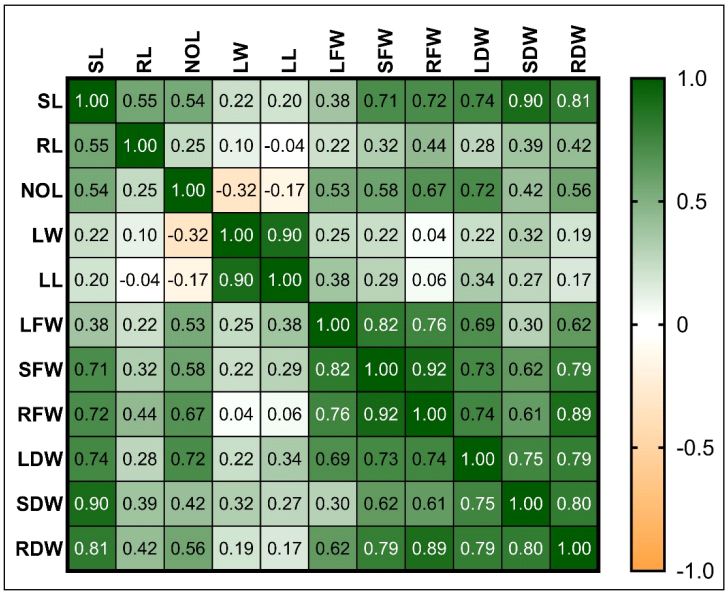
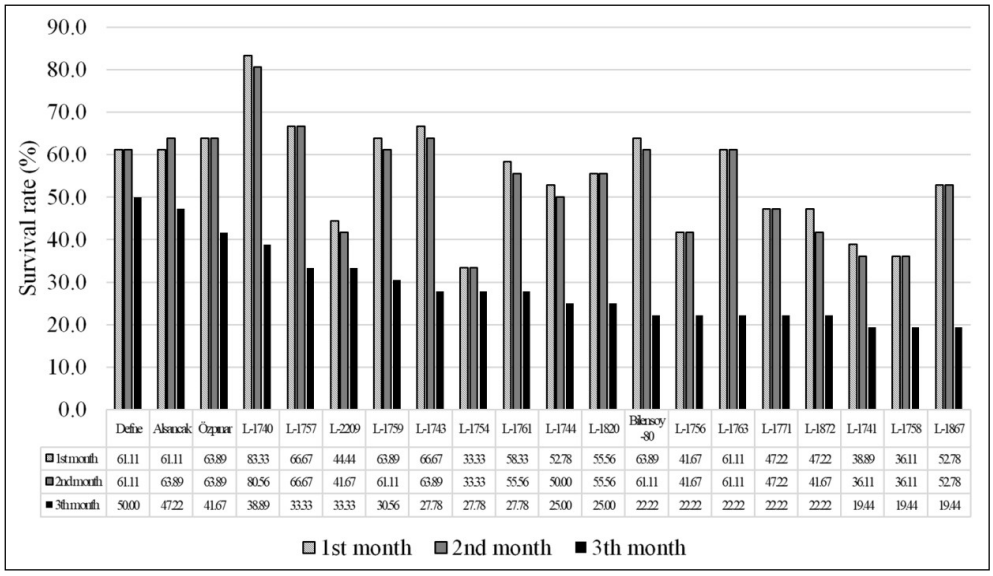


Fig 1: Correlation coefficient of the examined characters in alfalfa under high-salt stress.

Table 3: The percentages of crude ash, sodium and chloride ions in alfalfa populations and cultivars under salt stress.

Population/Cultivar	Ash (%)		Na^+ (%)		Cl^- (%)	
	Control	Salt	Control	Salt	Control	Salt
Defne	20.90	15.57	0.16	3.65	1.98	8.08
Alsancak	22.00	15.40	0.16	4.49	1.85	6.30
Özpınar	23.49	15.95	0.16	4.08	1.75	7.43
Bilensoy-80	19.79	16.00	0.09	3.93	1.38	7.33
L-1740	19.42	15.13	0.12	4.52	1.63	9.93
L-1757	18.71	15.97	0.11	3.99	1.45	5.73
L-2209	21.39	13.57	0.16	3.36	1.50	6.76
L-1759	20.13	14.55	0.10	3.76	1.43	6.27
L-1743	20.34	15.53	0.11	4.58	1.43	9.33
L-1754	16.17	15.06	0.14	4.41	1.53	7.53
L-1761	21.61	16.18	0.16	5.23	1.40	7.05
L-1744	13.83	18.12	0.25	4.76	1.40	6.75
L-1820	17.87	14.47	0.11	3.69	1.70	4.68
L-1756	16.55	15.63	0.11	3.72	1.93	6.72
L-1763	14.56	18.27	0.17	6.87	1.70	11.50
L-1771	13.86	18.45	0.13	6.24	1.78	10.40
L-1872	18.96	17.74	0.12	4.92	2.05	8.20
L-1741	16.90	14.02	0.14	5.18	1.83	8.01
L-1758	13.47	18.48	0.14	4.83	1.73	8.05
L-1867	14.37	15.55	0.14	5.86	1.53	9.77

according to climate in different seasons. They found that leaf width and length values ranged from 0.6-1.7 cm and 0.6-2.4 cm, respectively. In this study, we observed that the size of the first true leaf increased as the stress duration under high salinity increased. In the control group, the growth increase was less pronounced and rapid and the plant expended less energy in producing the first leaf. It is likely that the energy expended for plant growth during vegetative development is used for different tissues/organs of the plant, as the plant does not experience stress in this group. Under salt stress, morphological findings such as shoot length, root length and wet and dry weights of leaves, stems and roots of alfalfa generally decrease. However, short-term stress can sometimes have a positive effect on plant growth. Sandhu *et al*. (2017) reported that salinity caused a 12-34% decrease in shoot length in various alfalfa genotypes compared to the control. Similarly, Tani *et al*. (2018) found that the height of alfalfa seedlings decreased by approximately 50% when exposed to salt shock. Safarnejad *et al*. (1996) described how high salinity reduced the root length of alfalfa seedlings by up to 50%. Monirifar (2008) found that salt stress can cause up to 90% leaf loss in alfalfa. Under high salt stress, Wang *et al*. (2009) concluded that root fresh weight decreased by 32.17% and 78.32% in different alfalfa cultivars. Valizadeh *et al*. (2013) noted a 33.7% and 34.7% decrease in leaf and stem dry



	Defne	Alsancak	Özpınar	L-1740	L-1757	L-2209	L-1759	L-1743	L-1754	L-1761	L-1744	L-1820	Bilensoy-80	L-1756	L-1763	L-1771	L-1872	L-1741	L-1758	L-1867
1st month	61.11	61.11	63.89	83.33	66.67	44.44	63.89	66.67	33.33	58.33	52.78	55.56	63.89	41.67	61.11	47.22	47.22	38.89	36.11	52.78
2nd month	61.11	63.89	63.89	80.56	66.67	41.67	61.11	63.89	33.33	55.56	50.00	55.56	61.11	41.67	61.11	47.22	41.67	36.11	36.11	52.78
3th month	50.00	47.22	41.67	38.89	33.33	33.33	30.56	27.78	27.78	27.78	25.00	25.00	22.22	22.22	22.22	22.22	22.22	19.44	19.44	19.44

Fig 2: Survival rates of alfalfa populations and cultivars under salt stress.

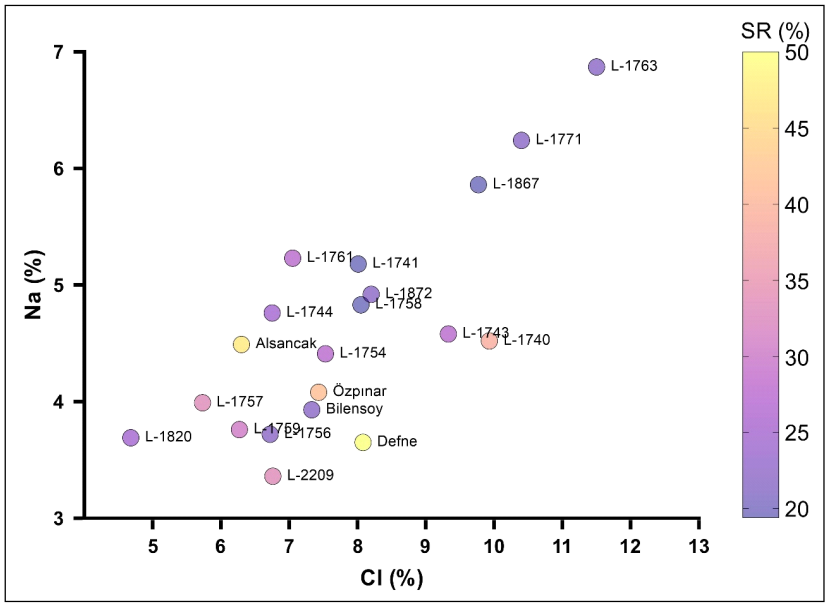


Fig 3: Sodium (Na^+), chloride (Cl^-) contents and survival rates of alfalfa cultivars and populations under salt stress.

weight, respectively, due to salt stress. Arab and Ehsanpour (2013) detected that under salt stress, the root dry weight of alfalfa decreased by more than 60%. However, Wang *et al*. (2012) found that short-term salt stress in alfalfa increased root dry weight by 45.83% by stimulating root growth. In this study, we observed that the decrease in the mentioned parameters increased with the severity and duration of stress in alfalfa genotypes. In tolerant plants that adapt to stress conditions, growth does not stop and plants continue to develop, albeit slightly. The differences in reduction rates can be attributed to the duration and severity of salt stress, as well as to genetic variations.

Correlation coefficient is a popular tool used to analyze data in agricultural research (Kozak, 2012). Strbanovic *et al*. (2017) investigated the correlation between yield and quality traits in alfalfa, while Wang *et al*. (2020) examined the correlation between survival rate, fall dormancy and yield in alfalfa. The correlation analysis revealed that among the morphological parameters examined in stressed plants, shoot length (SL) was highly correlated with yield related parameters (SFW, LFW, SDW and LDW). This feature can be used as a selection criterion in the selection of tolerant individuals.

As soil salinity increases, particularly due to improper irrigation practices, the mineral content of the soil solution also increases. This, in turn, is expected to increase the amount of minerals that plants absorb. Avcı (2018) determined that the amount of crude ash in alfalfa increased by 11.05%, 15.5% and 16.25%, respectively, in a linear relationship with increasing salinity stress levels (0.25, 1.50 and 3.00 dS m^{-1}). In plants, high salinity generally causes sodium (Na) and chloride (Cl) ions to accumulate and K ions to decrease (Gupta and Huang, 2014). Several studies have highlighted an increase in Na^+ and Cl^- ions and a decrease in K ions in plant organs such as leaves and stems of alfalfa plants being exposed to NaCl salt stress (Arab and Ehsanpour, 2006; Ai-Ke *et al*., 2009; Campanelli *et al*., 2013). The plant nutrients that can be utilized under saline conditions also depend on the genetic structure of alfalfa. Ferreira *et al*. (2015) observed that exposure to salt stress resulted in an increase of Na^+ and Cl^- ions and a decrease of K and Ca ions in alfalfa shoots. It was found that salinity levels had a positive correlation with the increase of nitrogen, phosphorus, magnesium and total sulphur in shoots. Sandhu *et al*. (2017) reported that under high salinity, sodium concentrations in alfalfa of different genotypes tended to increase up to 2.5-4.6 times compared to the control. However, under saline conditions, Cl concentration in shoots increased in addition to an increase in Mn and Zn and a decrease in Ca, Mg, P, Fe and Cu for most genotypes. In this study, it was observed that Na^+ and Cl^- ions increased under stress in all genetic materials under high salinity conditions. However, crude ash content increased in L-1744, L-1763, L-1771, L-1758 and L-1867 populations compared to the control under stress, while it decreased in other populations/cultivars. Possible reasons for differences in plant nutrient uptake under stress may include variations in alfalfa's developmental stage and genetic variability. Under salt stress, plants that are tolerant exhibit lower concentrations of Na^+ and Cl^- in their structures compared to plants that are less tolerant (Noble *et al*., 1984). The study found that more tolerant materials had a lower capacity for uptake of Na^+ and Cl^- ions. Thus, the survival rate generally increased as the plant material absorbed lower amounts of sodium and chlorine. The survival of alfalfa plants that exhibit tolerance to high saline conditions varies depending on the level and duration of stress, genetic structure and developmental stage of the plant. Towards the end of the third month, the survival of existing alfalfa populations/cultivars decreased under high salt conditions. Genetic diversity caused by allogamous plants in alfalfa and the effect of stress level and duration on plants able to adapt to high salinity are the main factors contributing to this situation.

CONCLUSION

Alfalfa can enhance its tolerance towards salt stress by reducing vegetative growth and minimizing the uptake of Na^+ and Cl^- ions into its structure. However, some tolerant individuals may continue to grow and develop, albeit at a slower pace. In tolerant populations or cultivars, there may be variability in the degree of resistance to high salinity over time. It could be recommended to use shoot length as a selection criterion for identifying tolerant individuals. In plant breeding, it is essential to have genetic material that shows resistance to long-term high salt stress. This is crucial for developing varieties that are suitable for cultivation under field conditions. This study revealed that alfalfa genotypes with a certain degree of salt tolerance showed variability in their tolerance to long-term salt stress. These findings indicate that long-term stress tests will give more reliable and precise results than short-term studies in order to select genotypes that will be exposed to continuous salt stress under field conditions. To ensure reliable performance, the material's characteristics and performance must undergo rigorous testing under field conditions.

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Conflict of interest

All authors declare that they have no conflict of interest associated with this study.

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