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THE EFFECTS OF ETHYL METHANESULFONATE APPLICATIONS ON CONTENTS OF MACRO AND MICRO ELEMENTS IN SUNFLOWER (*HELIANTHUS ANNUUS* L.) CULTIVATED *IN VITRO* UNDER SALINE CONDITIONS

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Abstract

Salt tolerant cultivars of sunflower can be obtained from induced mutation. The objective of the present study was to induce mutation for salt tolerance using ethylmethanesulphonate (EMS) in sunflower, followed by plant regeneration in *In vitro* under saline conditions and determine the effect of salinity stress on macro and micro plant nutritions of the leaves of two sunflower cultivars (Çetin Bey and Palancı I). Kernel of seed of "Çetin Bey" and "Palancı I" cultivars were kept in EMS solution at different doses (0, 0-1, 0-1, 5% and 2,0%) and taken into *In vitro* culture containing NaCl at different doses (0,0-1,25-2,50 and 5,00 g/L). The response of the cultivars used in the research on minerals showed differences. Although the effect of increasing NaCl doses on mineral content varied, mineral content, in general, was found to be high at 2,5 g/L NaCl dose. The effect of applied EMS doses on N, P and Zn contents was insignificant, and 1,0% EMS applications had a positive effect on the amount of other mineral substances, causing an increase. The study concluded that EMS applications could have a positive effect on the development of sunflower under saline conditions.

Key words: Sunflower, *In vitro*, Ethyl methanesulfonate, Salinity conditions.

Introduction

Salinity is an event where soluble salts are washed and mixed into groundwater and come to the surface of the soil with high base water followed by the accumulation of these salts on the soil surface as a result of water being reduced through evaporation (Bayat *et al.*, 2014). Soil salinity (Sha *et al.*, 2019) or water salinity is one of the leading stress factors and can adversely affects plant production (Shannon, 1998). Salinity may occur as a result of natural factors and unsuitable agricultural practices (Kalaji & Pietkiewicz, 1993) and although it causes significant product losses every year, stress factors cause about 25% loss of products per year (Gill *et al.*, 2004).

In plants with salt tolerance, salt is not taken into the plant, stored in intracellular spaces without being inserted into physiological events, deported from the plant, so the tissue tolerance and antioxidant substances are provided, and furthermore, some of the ions such as K^+, Na^+, Cl^- and $SO4^=$ are not carried into the plants or scapus (Parida & Das, 2005; Iqbal *et al.,* 2001). There are significant differences between plants in terms of tolerance to salt, regardless of the genus, species, genotypes or even organs (Belkhodja *et al*., 1994; Maas & Hoffman, 1977).

Some applications are being carried out to prevent salinity or to reclaim salty soils. In addition, studies aimed at discovering or developing genetically salt-resistant plant cultivars are gaining importance.

One of the most important oil plants of today is the sunflower plant. Sunflower is in the first place among vegetable oils, and sunflower seed oil is preferred for food quality. In the world, 11% of vegetable crude oil production is obtained from sunflower. Sunflower is consumed as a snack except for the consumption of oil. Confectionery sunflower seeds are rich in vegetable proteins and are widely used (Saltalı & Yıldırım, 2016).

The absence of tolerant cultivars against environmental stresses leads to considerable loss of product and quality when breeding in adverse conditions. Increasing yield in sunflower farming is possible with efficient and high-quality seed usage. The absence of resistant cultivars in the sunflower against stress factors causes yield to fall (Fernández-Moroni *et al.,* 2012). With increasing population in the world, nutrition arises as a problem. Nutrition is considered as the most important requirements for the survival and sustainable proper functions of organisms. Today, human health and animal feeding are even more prominent the interest in high nutritional products is increasing steadily (Sönmez *et al.*, 2018). Oil, which is the most important food source, and confectionery sunflowers are two of the most important industrial foods of today, and sunflower is cultivated in large areas. It is important to determine the salt resistance of sunflower cultivars that are produced today and to investigate the cultivars that will yield at economical levels. Some salt-resistant cultivars were determined using tissue culture method (Al-Jibouri *et al.*, 2005). However, very little information is available about sunflowers resistance to salt stress.

In addition to classical breeding methods, mutations are also utilized in plant breeding studies. Materials that promote mutation are named mutagens. It is divided into two groups as a physical mutagen and chemical mutagen (Lestari, 2012). Mutation is defined as a process that causes changes in genetic material through chemical or physical mutagens (Altındal & Altindal, 2018). The ability of chemical mutagens to create a specific and possible mutation is greater (Greene *et al.,* 2003). Ethyl methanesulphonate (EMS) is in the most commonly used chemical mutagens (Krupa-Małkiewicz *et al.*, 2017). EMS is a toxic chemical that changes the chemical structure of nucleotides by interacting with DNA. EMS is used in the mutation of developing cells by adding alkyl groups to the nucleotides in DNA and changing their sequence and nucleotide match properties (Waugh *et al.*, 2006) which may cause deletion in some small DNA regions and re-formations in other chromosomes (Greene *et al.*, 2003). EMS can be applied in *In vitro* explants of many species (Duron, 1992) and can be uniformly treated to cells.

To create variations in properties such as tolerance to salinity in plants, plant breeders use the mutation method, and this method is an alternative to classical breeding methods. The genetic diversity, resulting from mutations created by various mutagens, contributes to the aims of plant breeders. As in all of plant species, it is necessary to develop new cultivars in sunflower and accelerate the breeding programmes.

Salt tolerant cultivars of sunflower can be obtained from induced mutation. In this study, the effects of different EMS doses on salt tolerance in sunflower cultivars were examined. It could be confirmed that measuring macro and micro nutrients of leaves can be used to selection criterion for developing sunflower salinity tolerance cultivars. In addition, we attempted to create genetic variation using ethyl methanesulfonate for salt tolerance of sunflower, thus we aimed to provide a new material source to breeders, especially for breeding studies.

Materials and Methods

The study was established with 10 replications to determine the effect of cultivar, NaCl and EMS doses on the intake of plant nutrients of sunflower in *In vitro*, and the results were analyzed according to the factorial experiment design in randomized plots. In the study, the nutrition media were prepared using stock solutions of micro and macro elements of the basic MS medium (Murashige & Skoog, 1962) to create plant regeneration from the kernel of seed exposed to the EMS (Ethyl methanesulfonate-SIGMA-molecular weight: 124.16; density: 1.206). Nutrition media (MS) was supplemented with 30 g/L sucrose as a source of carbon and 8 g/L agar (Hassan *et al.*, 2004). Then the pH was adjusted to 5.8, the prepared media were sterilized at 121 ºC for 15 minutes. In addition to the basic MS medium for plant regeneration, the hormones (0.1 mg GA_3, 0.01 mg NAA and 0.01 mg kinetin) and NaCl (0,0-1,25-2,50-5,00 g/L NaCl) as stress factor were used. The water-soaked seeds of sunflower cultivars were kept in a 15% sodium hypochlorite solution (1-2 drops of 1% Tween-20 added) for 20 minutes in the sterile cabin for surface sterilization and washed 5 times with sterile water. Subsequently, the sterilized kernels of seeds were waited for 90 min in EMS solutions at different doses (0,0-1,0-1,5% and 2,0%) and then washed with sterile pure water 3-5 times. EMS applied explants were cultured in growth medium (MS) containing NaCl for plant regeneration, for 4 weeks in 16 hours light and 8 hours dark, at 2000-2200 lux light and 25°C. Fe, Ca, Mg, Cu, Mn and Zn levels were measured with atomic absorption spectrophotometer in the leaves belonging to plants regenerated in *In vitro* (Uygur & Şen, 2018; Kaçar, 1972). N content was determined by Kjeldahl, as well as, K content by Flamephotometric method and P content by Molibdovanado-phosphoric acid methods (Kaçar, 1972). The resulting values were analyzed in the SAS package software. Significant differences were determined by the multiple comparison tests of LSD and Duncan in the MSTAT-C software.

Nitrogen (N) content (%): The effect of cultivar on sunflower N ratio was very important in the *In vitro* study, and the highest N ratio was determined in "Çetin Bey" cultivar (5,25%) (Table 1).

The interaction of the cultivar x NaCl dose was statistically significant (p<0.01) (Table 1), and N ratios ranged from 4,99% to 5,32. In general, the responses of the cultivars to the NaCl dose were different, there were no differences between the control groups and cultivars, and the N ratio of the "Çetin Bey" cultivar increased after the applications compared to the control group, and the highest N ratio was 5,32% at 1,25 g/L NaCl dose. Lowest N ratios were obtained from the "Palancı I" cultivar.

The interaction of the cultivar x EMS dose had a significant effect on N ratio (p<0.01), while the N levels of cultivars changed according to the EMS doses. "Çetin Bey" gave the best response to EMS applications. While the lowest N ratio was obtained from "Palancı I" with 1,0% EMS dose (4,96), the highest N ratio was obtained from "Çetin Bey" at the same dose (5,42%), and the responses of the cultivars were different. There was no significant increase in the ratio of N in the "Palancı I" cultivar with EMS applications.

Cultivar x NaCl dose x EMS dose interaction was statistically very significant (p<0.01) (Table 1). The lowest N ratio was obtained from the "Palancı I" with 0,0 g/l NaCl and 1,0% EMS application (4,67%) and the highest was obtained from "Çetin Bey" with 2,5 g/L NaCl and 1,0% EMS (5,62%) application (Table 1).

Phosphorus (P) content (%): The effect of the cultivar on P ratio was significant (p<0.01), and P ratio was found higher in the "Palancı I" cultivar (0,62%). In addition, the interaction between the cultivar and the NaCl dose was also significant (p<0.01).

There was a difference between the responses of the cultivars to NaCl doses. The 5,0 g/L NaCl application significantly reduced the P ratio in "Çetin Bey" cultivar compared to the control group, while the "Palancı I" cultivar did not cause any significant differences. In addition, there were no significant differences between NaCl applications in the "Palancı I" cultivar (Table 2).

The response of the cultivars to EMS applications was insignificant, and P ratios varied between 0,57% and 0,65%. Cultivar x NaCl dose x EMS dose interaction was statistically significant (p<0.01). However, the response of the cultivars to the EMS doses was non-significant, while significant differences in P ratio emerged as a result of cultivar x NaCl dose interaction. The lowest P ratio was obtained in the "Çetin Bey" cultivar in 5,0 g/l NaCl and 1,0% EMS application (0,50%), and the highest was obtained from the "Palancı I" in 2,5 g/L NaCl + 1,0% EMS application (% 0,70) (Table 2).

Table 1. Nitrogen (N) content of the plants obtained from sunflower cultivars for different NaCl and EMS dosage applications (%).

Cultivar	**NaCl doses (g/L)**	**EMS doses (%)**				
		Control	**1,0**	**1,5**	**2,0**	**Mean**
Çetin Bey	Control	5,05 e-ı	5,33 a-e	4,97 f-ı	5,23 b-g	5,15 **CD**
	1,25	5,35 a-e	5,37 a-d	5,33 a-e	5,24 b-g	5,32 **A**
	2,50	5,30 b-e	5,62 a	5,15 c-ı	5,13 c-ı	5,30 **AB**
	5,00	5,40 abc	5,35 a-e	5,11 c-ı	5,08 d-ı	5,23 **ABC**
	Mean	5,28 **b**	5,42 **a**	5,14 **c**	5,17 **bc**	5,25 *a*
Palancı I	Control	5,22 b-g	4,67 j	5,17 b-h	5,47 ab	5,13 **CD**
	1,25	5,09 c-ı	4,87 hıj	4,85 ıj	5,12 c-ı	4,99 **E**
	2,50	4,94 g-j	5,26 b-f	5,24 b-g	5,25 b-g	5,17 **BCD**
	5,00	5,14 c-ı	5,04 e-ı	5,26 b-f	4,68 j	5,03 **DE**
	Mean	5,10 **c**	4,96 **d**	5,13 **c**	5,13 **c**	5,08 *b*
	Control	5,13 CD	5,00 DE	5,07 CDE	5,35 AB	5,14 ***B***
	1,25	5,22 BC	5,12 CD	5,09 CD	5,18 BCD	5,15 ***B***
	2,50	5,12 CD	5,44 A	5,20 BCD	5,19 BCD	5,24 ***A***
	5,00	5,27 ABC	5,19 BCD	5,19 BCD	4,88 E	5,13 ***B***
	Mean	5,19	5,19	5,14	5,15	

LSD = NaCl dose (N): 0,06981* **EMS dose (E):** Non-significant

Italic lowercase: Cultivar; Italic bold uppercase: NaCl dose; Bold uppercase: Cultivar x NaCl dose; Bold lowercase: Cultivar x EMS dose; Uppercase: NaCl dose x EMS dose; Lowercase: Cultivar x NaCl dose x EMS dose

Table 2. Phosphorus (P) content of the plants obtained from sunflower cultivars for different NaCl and EMS dosage applications (%).

Cultivar	**NaCl doses (g/L)**	**EMS doses (%)**				
		Control	**1,0**	**1,5**	**2,0**	**Mean**
Çetin Bey	Control	0,62 a-e	0,66 abc	0,60 a-e	0,58 a-e	0,61 **AB**
	1,25	0,63 a-d	0,54 cde	0,63 a-d	0,59 a-e	0,60 **AB**
	2,50	0,53 de	0,62 a-e	0,53 de	0,62 a-e	0,57 **BC**
	5,00	0,61 a-e	0,50 e	0,53 de	0,52 de	0,54 **C**
	Mean	0,60	0,58	0,57	0,58	0,58 *b*
Palancı I	Control	0,64 a-d	0,53 de	0,64 a-d	0,69 ab	0,62 **AB**
	1,25	0,57 b-e	0,57 b-e	0,56 cde	0,66 abc	0,59 **ABC**
	2,50	0,60 a-e	0,70 a	0,63 a-d	0,63 a-d	0,64 **A**
	5,00	0,64 a-d	0,61 a-e	0,62 a-e	0,62 a-e	0,62 **AB**
	Mean	0,61	0,60	0,61	0,65	0,62 *a*
	Control	0,63 AB	0,59 AB	0,62 AB	0,63 AB	0,62 ***A***
	1,25	0,60 AB	0,55 B	0,59 AB	0,63 AB	0,59 ***BC***
	2,50	0,56 B	0,66 A	0,58 AB	0,63 AB	0,61 ***AB***
	5,00	0,62 AB	0,56 B	0,58 AB	0,57 B	0,58 ***C***
	Mean	0,60	0,59	0,59	0,61	

LSD = NaCl dose (N): 0,02693* **EMS dose (E):** Non-significant

Italic lowercase: Cultivar; Italic bold uppercase: NaCl dose; Bold uppercase: Cultivar x NaCl dose; Uppercase: NaCl dose x EMS dose; Lowercase: Cultivar x NaCl dose x EMS dose

Potassium (K) content (%): The effects of cultivars on potassium ratio were important in the plants obtained from the kernel of seeds of sunflower ($p<0.01$). On an average, the cultivars of "Çetin Bey" and "Palancı I" were 1,79% and 1,25% K, respectively (Table 3).

Cultivar x NaCl dose and cultivar x EMS dose interactions as bilateral interactions were found to be non-significant. In both cultivars, a high amount of K ratio was obtained at the 5.0 g/L NaCl doses. The response of the cultivars to the EMS doses was different, and EMS applications caused to increase in K ratios. The highest K ratio was observed at 1,0% and 1,5% EMS doses in "Çetin Bey" cultivar, while it was obtained from the "Palancı I" cultivar at 2.0% EMS dose (Table 3).

Although the response of the K ratio of the cultivars on the doses of NaCl and EMS was non-significant, the effect of the trilateral interaction (cultivar x NaCl dose x EMS dose) on the K ratio was found to be significant ($P<0.05$) (Table 3), and the values varied between 0,87-2,17%. The 2,5 and 5,0 g/L NaCl dose in the "Çetin Bey" cultivar resulted in high K ratios, which were similar to EMS applications.

Calcium (Ca) content (%): The effect of sunflower cultivars on the Ca ratios was found to be statistically significant ($p<0.01$), and the maximum Ca ratio was obtained in "Çetin Bey" cultivar with 0,31% (Table 4).

According to the bilateral interactions, e.g. cultivar x NaCl dose and cultivar x EMS dose, the response of cultivars to NaCl and EMS doses in terms of the Ca ratio was non-significant. However, the highest Ca ratios emerged in "Çetin Bey" cultivar, and these ratios were determined at 1,25 g/L NaCl dose (0,32%) and 1,0% EMS dose (0,33%) (Table 4).

According to the trilateral interaction of the factors discussed in the experiment, the interaction of the cultivar x NaCl dose x EMS dose was significant (Table 4), while the Ca ratio ranged from 0.11-0,36 (Table 4). The highest Ca ratio in the "Çetin Bey" cultivar was 0.36% at 0,0 g/l NaCl + 1,0% EMS doses; 1,25 g/L NaCl + 1,5% EMS doses; and 2,5 g/L NaCl + 0,0% EMS doses. The highest Ca ratio in the "Palancı I" was 0,24% at 0,0 g/L NaCl + 0,0% EMS and 0,0 g/L NaCl + 1,0% EMS doses (Table 4).

Magnesium (Mg) content (%): The effect of the cultivar on the ratio of the Mg was not significant, and the Mg ratio of both cultivars was 0,32% (Table 5).

The interaction of cultivar x NaCl dose was significant ($p<0.05$) (Table 5) and Mg ratios ranged between 0,29% and 0,36. There was no significant difference between the NaCl applications in "Çetin Bey"cultivar. The highest Mg ratio was obtained in both cultivars at 2,5 g/L NaCl dose, and there was no significant difference between them.

The response of the cultivar to EMS doses was found to be non-significant, and the Mg ratios varied between 0,31% and 0,35% (Table 5).

The effect of cultivar x NaCl dose x EMS dose interactions on Mg ratio was non-significant, although the maximum Mg ratio emerged at 2,5 g/L NaCl+1,0% EMS doses in the "Palancı I" cultivar (0,39%) (Table 5).

Iron (Fe) content (mg/kg): The cultivar factor had significant effect on the amount of Fe ($p<0.01$), and the response of the "Çetin Bey" cultivar to the amount of Fe was more. The amount of Fe was 140,39 mg in "Çetin Bey" and 101,03 mg in the "Palancı I" (Table 6).

According to the bilateral interactions of the factors applied in the experiment, the response of both cultivars on the Fe content was different and the amount of Fe was significantly decreased as the NaCl dose increased in the "Palancı I", and the highest values were obtained from 0,0 g/L and 1,25 g/l NaCl applications (138,85 and 113,95 mg respectively). The effect of NaCl doses on Fe amount was similar in "Çetin Bey" cultivar (excluding 5,0 g/L NaCl), the highest value was observed as 1,25 g/l NaCl dose (Table 6).

The response of the cultivars to EMS doses was significant, and the interaction of the cultivar x EMS dose was found statistically significant ($p<0.01$). The values of Fe changed between 88,20-152,90 mg. The effect of 2,0% EMS caused similar results in both cultivars, but responses in other applications varied according to cultivars. The maximum Fe ratio in the "Çetin Bey" cultivar was obtained at 1,0% EMS (152.90 mg), it was achieved in the "Palancı I" at 2,0% EMS dose (108,05 mg) (Table 6).

In the study, the interaction between NaCl dose and EMS dose was found to be non-significant, and the amounts of Fe changed between 87,30-159,10 mg, the highest value was obtained from the 0,0 g/L NaCl+1,0% EMS application (Table 6).

Cultivar x NaCl dose x EMS dose interaction was significant ($p<0.05$). The lowest amount of Fe (58,10 mg) was obtained from the 2,5 g/L NaCl and 1,5% EMS application, the highest was obtained from the "Çetin Bey" with 0,0 g/L NaCl and 1,0% EMS application (174,80 mg) (Table 6).

Copper (Cu) content (mg/kg): In the study, the effect of the cultivar on copper was not significant, but it was found higher in the "Palancı I" cultivar (14,34 mg) (Table 7).

The effects of the bilateral interaction of the factors on the Cu content were found significant. The response of the cultivars to the NaCl doses showed variability, Cu values were determined between 11,82-17,12 mg. The highest Cu content was obtained in the 0,0 g/L NaCl application for cultivars. As NaCl doses increase in both cultivars, numerical declines emerged in the amount of Cu. There was no significant difference in the response of each cultivar to the NaCl doses (except the control group), and they were found in the same group (Table 7).

The effect of EMS doses on the Cu quantity of cultivars was statistically significant ($p<0.01$), and the highest Cu content (15,80 mg) was obtained from the application of 0,0% EMS dose in the "Palancı I". The effect of EMS applications in the "Çetin Bey" cultivar was similar, and there was no significant difference between them (Table 7).

Cultivar x NaCl dose x EMS dose interaction affected the Cu content significantly ($p<0.05$) (Table 7). Highest Cu content (18,90 mg) was obtained from NaCl and EMS-free applications of the "Palancı I" cultivar, and the lowest (10,50 mg) was obtained from the "Palancı I" with 5,0 g/L NaCl+1,5% EMS (Table 7).

Table 3. Potassium (K) content of the plants obtained from sunflower cultivars for different NaCl and EMS dosage applications (%).

Cultivar	NaCl doses (g/L)	EMS doses (%)				
		Control	1,0	1,5	2,0	Mean
Çetin Bey	Control	1,28 ı-l	1,64 d-h	1,72 c-g	1,72 c-g	1,59
	1,25	1,76 b-f	1,54 e-ı	1,79 b-f	1,58 e-ı	1,67
	2,50	1,77 b-f	1,98 abc	1,90 a-d	1,93 a-d	1,90
	5,00	2,05 ab	2,17 a	1,91 a-d	1,83 b-e	1,99
	Mean	1,72	1,83	1,83	1,76	1,79 *a*
Palancı I	Control	0,87 n	0,97 mn	1,04 lmn	1,51 f-j	1,10
	1,25	1,04 lmn	1,09 k-n	1,07 k-n	1,38 h-k	1,15
	2,50	1,21 j-m	1,41 g-j	1,37 h-k	1,33 h-l	1,33
	5,00	1,29 ı-l	1,57 e-ı	1,63 d-h	1,21 j-m	1,43
	Mean	1,10	1,26	1,28	1,36	1,25 *b*
	Control	1,08 G	1,31 FG	1,38 EF	1,61 A-E	1,34 ***B***
	1,25	1,40 DEF	1,32 FG	1,43 C-F	1,48 C-F	1,41 ***B***
	2,50	1,49 B-F	1,69 ABC	1,64 A-E	1,63 A-E	1,61 ***A***
	5,00	1,67 A-D	1,87 A	1,77 AB	1,52 B-F	1,71 ***A***
	Mean	1,41 ***b***	1,55 ***a***	1,55 ***a***	1,56 ***a***	

LSD = NaCl dose (N): 0,1250** **EMS dose (E):** 0,1250**

Italic lowercase: Cultivar; Italic bold uppercase: NaCl dose; Italic bold lowercase: EMS dose; Uppercase: NaCl dose x EMS dose; Lowercase: Cultivar x NaCl dose x EMS dose

Table 4. Calcium (Ca) content of the plants obtained from sunflower cultivars for different NaCl and EMS dosage applications (%).

Cultivar	NaCl doses (g/L)	EMS doses (%)				
		Control	1,0	1,5	2,0	Mean
Çetin Bey	Control	0,23 f-j	0,36 a	0,32 abc	0,31 a-d	0,30
	1,25	0,35 a	0,30 a-e	0,36 a	0,26 c-g	0,32
	2,50	0,36 a	0,33 ab	0,28 b-f	0,28 b-f	0,31
	5,00	0,30 a-e	0,35 a	0,27 b-f	0,25 d-h	0,29
	Mean	0,31	0,33	0,31	0,27	0,31 *a*
Palancı I	Control	0,24 e-ı	0,24 e-ı	0,17 ıjk	0,22 f-j	0,22
	1,25	0,19 hıj	0,19 hıj	0,20 g-j	0,16 jk	0,19
	2,50	0,20 g-j	0,19 g-j	0,16 jk	0,16 jk	0,18
	5,00	0,19 g-j	0,18 hıj	0,17 ıjk	0,11 k	0,16
	Mean	0,21	0,20	0,18	0,16	0,19 *b*
	Control	0,23 BCD	0,30 A	0,25 ABC	0,26 ABC	0,26 ***A***
	1,25	0,27 ABC	0,25 ABC	0,28 AB	0,21 CD	0,25 ***AB***
	2,50	0,28 AB	0,26 ABC	0,22 BCD	0,22 BCD	0,24 ***AB***
	5,00	0,25 ABC	0,27 ABC	0,22 BCD	0,18 D	0,23 ***B***
	Mean	0,26 ***ab***	0,27 ***a***	0,24 ***bc***	0,22 ***c***	

LSD = NaCl dose (N):0,02095* **EMS dose (E):** 0,02784**

Italic lowercase: Cultivar; Italic bold uppercase: NaCl dose; Italic bold lowercase: EMS dose; Uppercase: NaCl dose x EMS dose; Lowercase: Cultivar x NaCl dose x EMS dose

Table 5. Magnesium (Mg) content of the plants obtained from sunflower cultivars for different NaCl and EMS dosage applications (%).

Cultivar	NaCl doses (g/L)	EMS doses (%)				
		Control	1,0	1,5	2,0	Mean
Çetin Bey	Control	0,32	0,34	0,30	0,29	0,31 **BC**
	1,25	0,34	0,28	0,34	0,32	0,32 **BC**
	2,50	0,34	0,34	0,32	0,34	0,33 **AB**
	5,00	0,32	0,31	0,30	0,28	0,30 **BC**
	Mean	0,33	0,32	0,32	0,31	0,32
Palancı I	Control	0,38	0,28	0,29	0,32	0,32 **BC**
	1,25	0,30	0,28	0,28	0,30	0,29 **C**
	2,50	0,37	0,39	0,32	0,34	0,36 **A**
	5,00	0,34	0,35	0,33	0,29	0,33 **B**
	Mean	0,35	0,33	0,31	0,31	0,32
	Control	0,35	0,31	0,30	0,31	0,31 ***B***
	1,25	0,32	0,28	0,31	0,31	0,30 ***B***
	2,50	0,36	0,37	0,32	0,34	0,35 ***A***
	5,00	0,33	0,33	0,32	0,28	0,32 ***B***
	Mean	0,34 ***a***	0,32 ***ab***	0,31 ***b***	0,31 ***b***	

LSD = NaCl dose (N):0,02752** **EMS dose (E):**0,02070*

Italic bold uppercase: NaCl dose; Italic bold lowercase: EMS dose; Bold uppercase: Cultivar x NaCl dose

Table 6. Iron (Fe) content of the plants obtained from sunflower cultivars for different NaCl and EMS dosage applications (mg/kg).

Cultivar	NaCl doses (g/L)	EMS doses (%)				
		Control	1,0	1,5	2,0	Mean
Çetin Bey	Control	130,30 b-g	174,80 a	155,90 abc	139,00 b-e	150,00**A**
	1,25	174,30 a	157,40 ab	148,80 abc	122,90 c-h	150,85 **A**
	2,50	145,80 a-d	135,90 b-f	143,80 a-d	125,10 b-h	137,65 **AB**
	5,00	126,00 b-h	143,50 a-d	108,70 e-j	114,10 d-ı	123,07 **BC**
	Mean	144,10 **ab**	152,90 **a**	139,30 **ab**	125,28 **bc**	140,39 *a*
Palancı I	Control	127,90 b-h	143,40 a-d	128,30 b-h	155,80 abc	138,85**AB**
	1,25	105,40 f-j	124,60 b-h	96,30 h-k	129,50 b-g	113,95 **C**
	2,50	98,90 g-k	84,60 ı-l	58,10 l	86,40 ı-l	82,00 **D**
	5,00	77,40 jkl	69,20 kl	70,10 kl	60,50 l	69,30 **D**
	Mean	102,40 **de**	105,45 **de**	88,20 **e**	108,05 **cd**	101,03 *b*
	Control	129,10	159,10	142,10	147,40	144,43 ***A***
	1,25	139,85	141,00	122,55	126,20	132,40 ***A***
	2,50	122,35	110,25	100,95	105,75	109,83 ***B***
	5,00	101,70	106,35	89,40	87,30	96,19 ***C***
	Mean	123,25 ***ab***	129,18 ***a***	113,75 ***b***	116,66 ***ab***	

LSD = NaCl dose (N): 13,03** **EMS dose (E):** 13,03**

Italic lowercase: Cultivar; Italic bold uppercase: NaCl dose; Italic bold lowercase: EMS dose; Bold uppercase: Cultivar x NaCl dose; Bold lowercase: Cultivar x EMS dose; Lowercase: Cultivar x NaCl dose x EMS dose

Table 7. Copper (Cu) content of the plants obtained from sunflower cultivars for different NaCl and EMS dosage applications (mg/kg).

Cultivar	NaCl doses (g/L)	EMS doses (%)				
		Control	1,0	1,5	2,0	Mean
Çetin Bey	Control	16,90 b	17,70 ab	16,00 b-e	14,50 c-h	16,27 **B**
	1,25	14,40 c-h	14,80 c-g	14,80 c-g	16,10 b-e	15,02 **C**
	2,50	12,80 hıj	13,80 f-ı	13,00 g-j	14,50 c-h	13,53 **D**
	5,00	12,80 hıj	12,30 ıjk	12,30 ıjk	12,20 ıjk	12,40 **EF**
	Mean	14,23 **bc**	14,65 **b**	14,03 **bc**	14,32 **bc**	14,31
Palancı I	Control	18,90 a	17,80 ab	17,50 ab	14,30 d-h	17,12 **A**
	1,25	14,60 c-h	16,20 bcd	15,00 c-f	16,30 bc	15,53 **BC**
	2,50	14,80 c-g	14,20 e-h	11,20 jk	11,30 jk	12,88 **DE**
	5,00	14,90 c-g	10,60 k	10,50 k	11,30 jk	11,82 **F**
	Mean	15,80 **a**	14,70 **b**	13,55 **bc**	13,30 **c**	14,34
	Control	17,90 A	17,75 AB	16,75 ABC	14,40 EF	16,70 ***A***
	1,25	14,50 EF	15,50 CDE	14,90 DE	16,20 BCD	15,28 ***B***
	2,50	13,80 EF	14,00 EF	12,10 G	12,90 FG	13,20 ***C***
	5,00	13,85 EF	11,45 G	11,40 G	11,75 G	12,11 ***D***
	Mean	15,01 ***a***	14,67 ***a***	13,79 ***b***	13,81 ***b***	

LSD = NaCl dose (N): 0,7722** **EMS dose (E):** 0,7722**

Italic bold uppercase: NaCl dose; Italic bold lowercase: EMS dose; Bold uppercase: Cultivar x NaCl dose; Bold lowercase: Cultivar x EMS dose; Uppercase: NaCl dose x EMS dose; Lowercase: Cultivar x NaCl dose x EMS dose

Manganese (Mn) content (mg/kg): The effect of cultivars on manganese content was significant (Table 8). The Mn contents of "Çetin Bey" and "Palancı I" cultivars were 210,16 mg and 152,25 mg, respectively (Table 8).

The effect of the determined NaCl doses on Mn was significant ($p<0.01$) and showed variability between 169,10 and 189,19 mg. The highest Mn content occurred in all doses except the 5,0 g/L NaCl dose, and there were no significant differences between them (Table 8).

The effect of the interaction between cultivar and NaCl dose on the Mn content was found to be significant ($p<0.01$) and the response of the cultivars to NaCl doses was variable. Indeed, there was no significant difference between the NaCl applications in the "Çetin Bey" cultivar, and the highest Mn content was obtained at 2,5 g/L NaCl dose. The highest values in the "Palancı I" were determined in the doses of 0,0 g/L and 2,5 g/L NaCl, and they took place in the same group. The effect of other doses to the Mn content was similar except the control group (Table 8).

The response of the cultivars to the EMS doses was insignificant, and the highest values were found in the "Çetin Bey" cultivar. In both cultivars, the EMS application of 1,0% increased the Mn content (Table 8).

The interaction of all factors with each other was statistically significant ($p<0.01$) (Table 8). The highest Mn content (243,30 mg) was obtained by the kernel of seed of "Çetin Bey" taken to the culture with 0,0% EMS and 1,25 g/L NaCl. In general evaluation of the factors discussed on Mn content in this study, especially the response of the "Çetin Bey" cultivar to the applications of NaCl was similar, but with the EMS application, their effects on Mn content can be said to change at a satisfactory level. In terms of Mn content, the response of the "Çetin Bey" cultivar to the EMS doses in the 2,5 g/l NaCl conditions and the response of the "Palancı I" cultivar in 5,0 g/L NaCl dose+EMS application were similar to each other (Table 8).

Zinc (Zn) content (mg/kg): The cultivars used in the research had significant effect ($p<0.01$) on the Zn content (Table 9). The positive response of "Çetin Bey" cultivar on the Zn content was more, and Zn content was identified as 126,72 mg (Table 9).

The interaction of the three factors was statistically significant ($p< 0.05$) (Table 9). Zn content was highest (184,20 mg) at the group with 1,25 g/L NaCl+1,5% EMS doses, as well as, at the group (155,90 mg) without NaCl and EMS.

In general, the Zn contents of both cultivars following NaCl and EMS applications were numerically different, but they were in the same group. The lowest Zn contents were obtained from "Palancı I" exposed to the 1,0% and 1,5% EMS combinations with 5,0 g/L NaCl, but no differences were detected (96,30 and 97,70 mg, respectively) (Table 9).

Table 8. Manganese (Mn) content of the plants obtained from sunflower cultivars for different NaCl and EMS dosage applications (mg/kg).

Cultivar	NaCl doses (g/L)	EMS doses (%)				
		Control	1,0	1,5	2,0	Mean
Çetin Bey	Control	186,60 c-h	242,20 a	198,90 b-f	180,40 d-j	202,03 **A**
	1,25	243,30 a	191,30 b-g	231,50 ab	180,80 d-ı	211,73 **A**
	2,50	240,90 a	223,46 abc	219,10 a-d	205,80 a-e	222,32 **A**
	5,00	207,70 a-e	241,50 a	192,10 b-g	177,00 d-j	204,58 **A**
	Mean	219,63	224,62	210,40	186,00	210,16 *a*
Palancı I	Control	181,50 d-h	182,70 c-h	170,60 e-k	170,60 e-k	176,35 **B**
	1,25	144,10 h-m	151,70 g-m	127,20 lm	159,60 f-l	145,65 **C**
	2,50	160,90 f-l	169,70 e-k	128,50 klm	154,40 g-l	153,37 **BC**
	5,00	145,50 h-m	138,30 ı-m	138,10 j-m	112,60 m	133,62 **C**
	Mean	158,00	160,60	141,10	149,30	152,25 *b*
	Control	184,05	212,45	184,75	175,50	189,19 ***A***
	1,25	193,70	171,50	179,35	170,20	178,69 ***AB***
	2,50	200,90	196,58	173,80	180,10	187,85 ***A***
	5,00	176,60	189,90	165,10	144,80	169,10 ***B***
	Mean	188,81 ***a***	192,61 ***a***	175,75 ***ab***	167,65 ***b***	

LSD = NaCl dose (N): 16,64** **EMS dose (E):** 16,64**

Italic lowercase: Cultivar; Italic bold uppercase: NaCl dose; Italic bold lowercase: EMS dose; Bold uppercase: Cultivar x NaCl dose; Lowercase: Cultivar x NaCl dose x EMS dose

Table 9. Zinc (Zn) content of plants obtained from sunflower cultivars in different NaCl and EMS dosage applications (mg/kg).

Cultivar	NaCl doses (g/L)	EMS doses (%)				
		Control	1,0	1,5	2,0	Mean
Çetin Bey	Control	155,90 ab	129,77 bcd	121,20 cd	119,50 cd	131,59
	1,25	131,80 bcd	110,10 cd	184,20 a	127,30 bcd	138,35
	2,50	125,20 bcd	122,70 bcd	120,80 cd	108,50 cd	119,30
	5,00	117,80 cd	134,30 bc	108,40 cd	110,00 cd	117,63
	Mean	132,68	124,22	133,65	116,33	126,72 *a*
Palancı I	Control	129,90 bcd	117,80 cd	126,50 bcd	118,20 cd	123,10
	1,25	105,60 cd	109,50 cd	106,20 cd	122,70 bcd	111,00
	2,50	113,90 cd	124,40 bcd	101,20 cd	107,10 cd	111,65
	5,00	108,00 cd	96,30 d	97,70 d	100,40 cd	100,60
	Mean	114,35	112,00	107,90	112,10	111,59 *b*
	Control	142,90 A	123,78 AB	123,85 AB	118,85 B	127,35 ***A***
	1,25	118,70 B	109,80 B	145,20 A	125,00 AB	124,68 ***A***
	2,50	119,55 B	123,55 AB	111,00 B	107,80 B	115,48 ***AB***
	5,00	112,90 B	115,30 B	103,05 B	105,20 B	109,12 ***B***
	Mean	123,51	118,11	120,78	114,21	

LSD = NaCl dose (N): 13,50** **EMS dose (E):** Non-significantv

Italic lowercase: Cultivar; Italic bold uppercase: NaCl dose; Uppercase: NaCl dose x EMS dose; Lowercase: Cultivar x NaCl dose x EMS dose

Discussion

In salt stress, the ion balance in the root region of the plant deteriorates, and in the increasing amount of Na intake, Na enters into competition with the intake of other mineral substances, causing nutritional deficiency. Therefore, the intake of some basic elements used in metabolic events is prevented (Bohra & Döffling, 1993). In high salt concentrations, Ca intake and transport in plants is reduced so that ion imbalance occurs (Huang & Redman, 1995). Due to ion accumulation and irregularities in the opening and closing of the stomata, the total amount of chlorophyll decreases and photosynthetic activity is decreased and plant growth is negatively affected (Aranda & Syvertsen, 1996).

In a study carried out in paddy, the effect of increasing Na on K intake in plant stem and leaf was not fully observed, but it was determined that increasing salt in roots reduced K intake (Cho *et al*., 1996).

Taban *et al.,* (1999) investigated the sensitivity to salt stress in corn cultivars through adding 0 and 68 mmol/kg NaCl to soil. They found that the amount of Mn increased in salt stress, also the content of K decreased, the amount of Fe, Zn and Cu showed variation according to cultivars. Özcan *et al.,* (2000) raised three chickpea cultivars (Canıtez-87, ILC-195/2, Damla) in soils containing 0 and 68 mmol/kg NaCl and stated that the Damla cultivars were less affected by salt than other cultivars. They found that proline, Na, Cl and P contents increased in the cultivars under salt stress but K contents declined. However, as a result of studies on salinity tolerance in melon, there are opinions that K and Ca ions are not very effective in determining salinity tolerance (Demir, 2009).

In stress conditions, the synthesis of abscisic acid increases in plant cells. As the amount of abscisic acid increases, water-insoluble starch forms and K+ ions decrease (Çırak & Esendal, 2006). In contrast to this situation, salt stress conditions increased K ion in the present study. In addition, our research showed that the response of the cultivars on minerals varied, and their effects on Mg and Cu contents were non-significant, and the amount of other minerals except P were found to be higher in "Çetin Bey" cultivar. In stress conditions, the ratio of K is higher, and therefore Mg intake is not affected, this situation is thought to be due to cultivar effect. Potassium (K) in high concentrations have a negative effect on the intake of Ca and Mg, and this effect varies according to plant cultivar (Fageria, 1983).

Although the effect of increased NaCl doses on mineral content varies, other mineral contents, excluding Fe and Cu, were found to be high at 2,5 g/L NaCl dose and decreased at 5,0 g/L NaCl dose. In general, the limit of salt tolerance was determined as 2,5 g/L.

Mutagenic applications have been successful in providing tolerance to abiotic stress factors such as salt tolerance in barley and cold tolerance in paddy (Nehnevajova *et al.,* 2007).

In a study conducted in millet (*Setaria Italica*), embryonic calli were treated with 0,5% EMS for 2,5 hours, and NaCl-resistant plants were successfully regenerated (Lu & Jia, 1994).

In a study on salt resistance of Sweet potato (*Ipomoea batatas* L.), the mutant lines were obtained by applying 0,5% EMS to the callus for 2 and 2.5 hours. Salt resistance of these lines (50 and 100 mm NaCl doses) were determined. In the study, the salt resistance of mutant plants was more than the controls. The 2.5-hour mutagenicity application was determined to be the most appropriate time for 0,5% EMS dose, which provides the highest frequency of mutation for salt tolerance (Luan *et al.,* 2007).

In our study, the effects of EMS doses on N, P and Zn doses were found to be non-significant. In general, 1,0% EMS applications to the amount of other mineral substances led to a positive increase, and high-dose EMS applications in NaCl salinity conditions in both cultivars were shown to cause positive increases in some mineral substances, especially in "Palancı I" cultivars.

Conclusion

Although studies conducted at the molecular level attempt to develop new plant cultivars tolerant to salinity, especially studies using mutagens in *In vitro* or *In vivo* remain insufficient. In our study, which is an *In vitro* culture study aiming to obtain salt-tolerant plants in sunflower; we determined that the examined properties vary significantly according to the applied factors. In general, we report that the "Çetin Bey" cultivar gives better responses in terms of salinity tolerance. Increased NaCl concentrations had a negative effect on the properties in general, but the administered EMS doses reduced or diminished the negative effects of NaCl.

According to the results of this research, the investigated features showed a lot of variability depending on the used factors, usually, 1,0% and 2,0% EMS dose were determined to reduce the negative effect of NaCl.

According to the study and literature search, it is necessary to conduct further research on the development of salt-tolerant plants by utilizing induced mutations in *In vitro* conditions.

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