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Dual effects of microwave and salinity stresses on growth and Na^+ and K^+ amounts of durum wheat plants

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ABSTRACT

The aim of our study was to determine the dual effect of microwave and salinity stresses on germination rate, root length (cm), shoot height (cm), root/shoot ratio, K+, Na+ amount (mg-1) and K^+/Na^+ ratio of Triticum durum Desf. Ç-1252 seeds and seedlings. Microwave 1 energy level (90W), 2 treatment periods (28,56 sec.) and various salinity concentrations were used in this study. For the evaluation of each energy level and saline treatment, 30 seeds in equal sizes were used. The results showed that salinity stress for 75, 150 and 225 mM resulted in a decrease in germination rate of seeds ($P<0.01$). The rate of germination increased significantly with the microwave treatment in the 150 and 225 mM NaCl+90(W) applications. When the NaCl treatments were individually examined with those of control group, it was found that the root lengths and shoot heights of the plants have decreased, but it was found to have increased with individual microwave treatment ($P<0.01$). Also root length and shoot height increased in 75 and 225 mM NaCl+90W (28 sec.) treatments ($P<0.01$). The root/shoot ratio was found to have increased in all treatments compared to the control group except for the 75 mM NaCl+90W (56 sec.) and 150 mM NaCl+90W (28 sec.) treatments. The Na amounts of plants tissue increased with salt treatments. The salt treatments increased the Na^+ amount significantly ($P<0.01$) while amount of K^+ decreased under low-level saline treatment (75 mM NaCl), but the saline treatments in higher amount (150 mM NaCl) came up with a significant increase compared to the control group.

Keywords Salinity stress. Microwave stress. Germination rate. Root/shoot ratio. K^+/ Na^+ ratio.

INTRODUCTION

The social and individual demands increasing in parallel with the increasing population has also increased the number of scientific studies in the literature. These studies aim to struggle against the harmful effects of climate changes as a consequence of global warming, also struggle against the decrease of the farmland, which is considered to be %10 of the whole world land.

Under stress conditions, the germination of seeds is affected by creating an external osmotic potential that prevents water uptake due to the toxic effects of Na^+ and Cl^- ions both during imbibition and seedling establishment [1]. Also can specific ion effects, nutritional imbalance or a combined effect of all these factors [2, 3].

Removal of soil salinity is economically very significant. However, the removal of the salinity is not an economical task to be fulfilled so easily. Therefore, growing the plants resistance to salinity or making treatments to make plants or seeds resistant to salinity are considered to be necessity. Can one of these treatments be microwave?

Low-intensity microwave radiation enhances germination, plant height, fresh weight and enzymatic activities [4, 5] and can protect seedlings of *Isatis indigotica* from enhanced UV-B damage [6]. Chen et al. [7, 8]. have also found that weak microwave can enhance tolerance of wheat seedlings to salt and osmotic stress. Qiu et al. [9] showed that the microwave radiation had a positive physiological effect on the growth and development of cadmium stressed seedlings.

Therefore, this study aims to investigate the effects of *Triticum durum* Desf Ç-1252, which has an economical value in Turkey on salinity resistance system of the plants. May microwave and salinity have a dual effect on K^+ and Na^+ amount, germination rate, root length and shoot height, root/shoot ratio? Can weak microwave improve the negative effects of salinity stress? What physiological changes happen when plant is exposed to both factors? Are these changes positive or negative?

MATERIALS AND METHODS

Plant material and microwave treatment

In this study, Siemens HF 12G240 five-stepped (800/600/360/180/90 W) microwave oven was used. As plant material, the seeds of *Triticum durum* Desf. "Ç-1252" genotype was used. Microwave 1 energy level (90W), 2 treatment periods (28,56 sn) and 3 salinity concentrations (75, 150, 225 mM NaCl) were used in determining some parameters.

Germination and determining some parameters

The seeds treated at 90W energy level for the durations mentioned above (28-56 sec) were then placed into the 9 cm petri pots which have 15 ml distilled water and 75, 150, 225 mM NaCI. The petri pots were kept in a dark under 25°C temperature till the germination happens (about seven days). At the end of this period, the rate of germination in all treatments were found and at the end of 7. day, the root lengths, shoot heights and root/shoot ratios of the plants were noted. Root length and shoot height of each plant were measured with a ruler.

Tissue Na^+ and K^+ analysis

The seeds kept at 90W energy level for 28 and 56 seconds in microwave, were then cultivated in the pots full of peat, and the pot was kept in the plant growth room for 16 hours day/ 8 hour night photoperiod, under 18- 20°C. To salinize the soil, 75 and 150 mM NaCI solutions were prepared, and these solutions were given to the plants once in every other 3 days. At the end of the 21st day, the Na^+ and K^+ analysis was carried out with the method mentioned below at the Research and Application Center for Geothermal Energy, Ground Water and Mineral Resources, Suleyman Demirel University.

The dried plant tissue (1000 mg) was powderized and then exposed to 2 ml H_2SO_4 under 150 °C. 0.5 ml H_2O_2 was added into the extract and kept under 240°C de for one hour. Then, another 0.5 ml H_2O_2 was added into the extract and kept under 240°C for 20 minutes. This step was repeated till the solution became clear. The solution was diluted with distilled water for 50 times, and Na^+ and K^+ amounts were determined with the use of atomic absorbtion spectrophotometer [10].

Statistical analysis

The experiment was established with a randomized complete block design, performed three times for each application group and SPSS 15.0 software programme was used for the statistical analysis. Means were compared with Duncan's multiple range test (Duncan's test).

RESULTS

To measure the effect of microwave and salinity stress dual or individually on germination, the rate of germination was determined on 30 seed with the use of various saline concentrations and at 90 W energy level for two different time periods (26-58 sec.). The seeds were kept on wet germination beds. The data obtained through measurements is presented in Table 1.

It was seen that the 90 W (28-56 sec.) microwave applied on the seeds in Ç-1252 genotypes and each NaCl treatment decreased the rate of germination compared to that of control group (0 mM NaCl) ($P<0.01$) (Table 1). This decrease was experienced most on the treatment of 90 W 56 sec. microwave. The rate of germination has decreased gradually with the increasing NaCl treatments ($P<0.01$) (Table 1).

The rate of 90% germination which was found in the 75 mM NaCl treatment (Fig. 1a) decreased in 75 mM NaCl+90W (28 and 56 sec.) treatment (Fig. 1b,c). The rate of germination which is 83.33% in 150 mM NaCl treatment (Fig. 2a) has led an increase by 86.66% in 150 mM NaCl+ 90W (28 sec.) (Fig. 2b) and also led an increase by similar rate to that rate in 225 mM NaCl treatment (Fig. 3b) (Table 1). The microwave treatment for 56 sec. was found to have decreased the rate of germination in both treatments ($P<0.01$) (Fig. 3bc).

Table 1. Effect of 90 W microwave (28 and 56 sec.) and various NaCl applications on germination rate, root length, shoot height and root/shoot ratio of Ç-1252 genotype seeds.

	Treatments	The number of germinated seeds	Germination rate (%)	Root length (cm)	Shoot height (cm)	Root/Shoot ratio
1	0 mM NaCl (control)	30^{a}	100,00^{a}	4.40^{c}	6.25^{ab}	0.69^{def}
2	75 mM NaCl	27^{b}	90,00^{b}	3.20^{cdef}	4.90^{c}	0.65^{def}
3	75 mM NaCl +90 W (28sec.)	26^{bc}	86,66^{bc}	6.10^{b}	5.55^{bc}	1.09^{def}
4	75 mM NaCl +90 W (56 sec.)	16^{f}	53,33^{f}	2.65^{defg}	4.95^{c}	0.53^{ef}
5	150 mM NaCl	25^{cd}	83,33^{cd}	4.05^{cd}	1.85^{d}	2.18^{c}
6	150 mM NaCl +90 W (28 sec.)	26^{bc}	86,66^{bc}	1.40^{g}	2.70^{d}	0.51^{f}
7	150 mM NaCl +90 W (56 sec.)	18^{e}	60,00^{e}	2.40^{efg}	1.15^{e}	2.08^{c}
8	225 mM NaCl	24^{d}	80,00^{d}	1.95^{fg}	0.85^{ef}	2.29^{c}
9	225 mM NaCl + 90 W (28 sec.)	25^{cd}	83,33^{cd}	3.80^{cde}	1.20^{e}	3.16^{b}
10	225 mM NaCl + 90 W (56 sec.)	18^{e}	60,00^{e}	2.25^{efg}	0.35^{f}	6.42^{a}
11	90 W (28 sec.)	25^{cd}	83,33^{cd}	9.60^{a}	7.00^{a}	1.36^{d}
12	90 W (56 sec.)	11^{g}	36,66^{g}	6.50^{b}	5.10^{c}	1.27^{de}

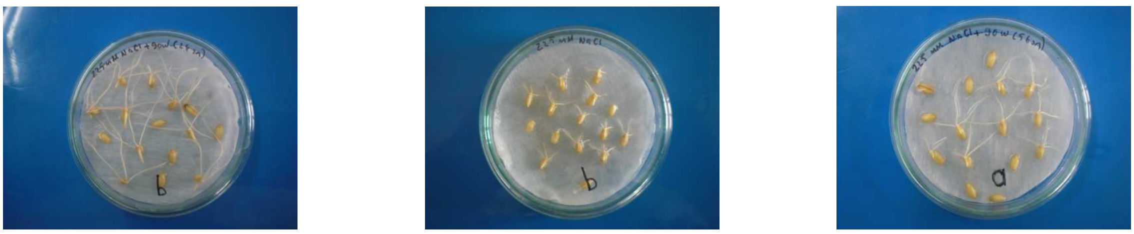
**The values that are followed by the same letter do not differ statistically at a significance level of 1% ($P<0.01$).*

a **b** **c**

Figure 1. Effect of 75 mM NaCl and 90W microwave treatment on germination of Ç-1252 seeds a)75 mM NaCl b) 75 mM NaCl+90W (28 sec.) c) 75 mM NaCl+90W (56 sec.)

a **b** **c**

Figure 2. Effect of 150 mM NaCl and 90W microwave treatment on germination of Ç-1252 seeds a)150 mM NaCl b)150 mM NaCl+90W (28 sec.) c) 150 mM NaCl+90W (56 sec.)



a **b** **c**

Figure 3. Effect of 225 mM NaCl and 90W microwave treatment on germination of Ç-1252 seeds a)225 mM NaCl b)225 mM NaCl+90W (28 sec.) c) 225 mM NaCl+90W (56 sec.)

Whereas the rate of germination was found to be 83.33% in 90W (28 sec.) treatment, it was found to be 86.66% in 75 mM NaCl+ 90W (28 sec.) and 150 mM NaCl+90 W (28 sec.) treatments, and it was found to be 83.33% in 225 mM NaCl+ 90 W (28 sec.) (Table 1). Whereas the rate of germination was 36.66% in 90W (56 sec.) treatment, it was found to be 53.33% in 75 mM NaCl+90 W (56 sec.), and 60% in 150 mM NaCl+90 W (28 sec.) and 225 mM NaCl+ 90 W (28 sec.) treatments ($P<0.01$) (Table 1).

When the NaCl treatments were individually examined with those of control group (0 mM NaCl), it was found that the root lengths and shoot heights of the plants have decreased, but it was found to have increased with individual microwave treatment ($P<0.01$) (Fig. 4).

In 75 mM NaCl treatment, the root length was found to be 3.20 cm and shoot height was found to be 4.90 cm. In the 75 mM NaCl+90W (28 sec.) treatment, the root length was found to be 6.10 cm, shoot height was found to be 5.55 cm, which means an increase in length ($P<0.01$) (Table 1). The saline + 56 sec. microwave treatment was found to have decreased the root length ($P<0.01$), the shoot height was found to have slightly increased. This increase was not found to be significant (Table 1).

In 150 mM NaCl treatment, the root length was found to be 4.05 cm, and the shoot height was found to be 1.85 cm. Whereas the 150 mM NaCl+90 W (28 sec.) treatment was found to have decreased the root length ($P<0.01$), the shoot height was found to have increased up to 2.70 cm (Table 2). However, this increase was not found to be significant. The saline + 56 sec. microwave treatment was found to have decreased both the root length and shoot height ($P<0.01$) (Fig. 4).

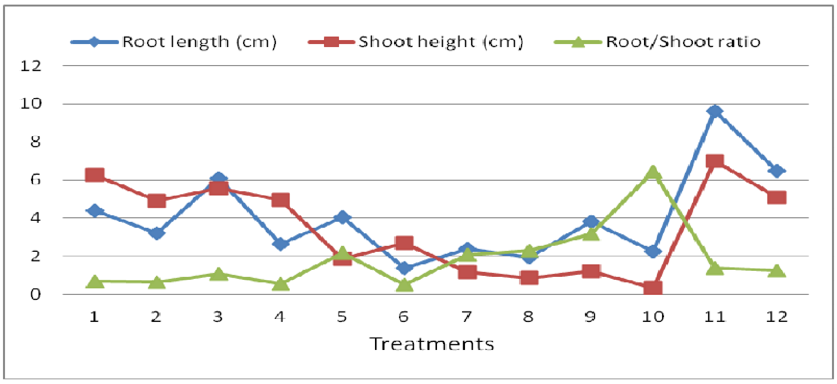


Figure 4. A diagram showing the effect of applications on root lengths, shoot heights and root/shoot ratios of seedlings

In 225 mM NaCl treatment, 28 sec. microwave was found to have increased the root length which was 1.95 cm, up to 3.80 cm ($P<0.01$), and it also increased the shoot height up to 1.20 cm ($P<0.01$) (Table 1). 56 sec. microwave treatment was found to have increased, but decreased the shoot height ($P<0.01$) (Fig. 4).

The single microwave treatments were found to have increased the root lengths and shoot heights of the plants compared to the control group. Whereas the root length was found to be 4.40 cm, the shoot height was found to be 6.25 cm in 0 mM NaCl control group, the root length in 90 W (28 sec.) treatment was found to have increased up to 9.60 cm, the shoot height was found to have increased up to 7.0 cm ($P<0.01$) (Table 1). The 56 sec. Microwave treatment was found to have slightly increased the root length, but have decreased the shoot height ($P<0.01$) (Fig. 4).

The ratio of root/shoot was found to be 0.69 in control group. Whereas this rate decreased down to 0.65 in 75 mM NaCl treatment, it was found to be 2.18 in 150 mM NaCl treatment ($P<0.01$) and 2.29 in 225 mM NaCl treatment ($P<0.01$) (Table 1). The root/shoot ratio which was found to have decreased in 75 mM NaCl+90 W (56 sec.) (0.53) and 150 mM NaCl+90 W (28 sec.) (0.51) treatment, compared to the control group ($P<0.01$) was found to have increased in all other treatments. The highest increase in root/shoot ratio was observed in 225 mM NaCl+ 90 W 28 and 56 sec. treatments ($P<0.01$) (Fig. 4).

The results of the Na^+ and K^+ analysis carried out on the plant tissue, suggest that the the Na^+ amount in the control grup was 1,49 mg/l, and the amount of K^+ was 23,54 mg/l . The saline treatments suggest that the Na^+ amount has increased (75 and 150 mM) ($P<0.01$). It also suggests that the amount of K^+ was found to have decreased under low-level saline treatment (75 mM NaCl) (this decrease is not statistically significant), the saline treatments in higher amount (150 mM NaCl) came up with a significant increase compared to the control group ($P<0.01$) (Table 2). In 75 mM NaCl+90 W (28 and 56 sec.) treatments, the amount of Na^+ was found to be significantly low compared to the 75 mM NaCl (P<0.01) (Fig. 5). In addition to this, the K^+ amount was found to have increased in 28 sec. treatment. However, this increase was not statistically significant. In 56 sec. treatment, the amount of K^+ has significantly increased (P<0.01) (Table 2). In the 150 mM NaCl treatment, the amount of Na^+ and K^+ in the 4,27 mg/l and 24.15 mg/l was found to have increased with 90 W-28 treatment (P<0.01), but it decreased with 90 W-56 sec. treatment (P<0.01) (Table 2).The amount of Na^+ and K^+ in both microwave treatments was found to have decreased compared to the control group (Fig. 5). However, in the 90 W (28 sec.) treatment, the decrease in the amount of K^+ was not significant.

Table 2. Effect of 90 W microwave (28 and 56 sec.) and various NaCl applications on Na^+ and K^+ amounts and K^+/Na^+ ratios in Ç-1252 genotypes leafs

	Treatments	Na^+ (mg/l)	K^+ (mg/l)	K^+/Na^+
1	0 mM NaCl (control)	$1,49^{bcd}$	$23,54^{abc}$	$14,75^{abc}$
2	75 mM NaCl	$10,79^{a}$	$16,73^{abc}$	$1,65^{c}$
3	75 mM NaCl+90 W (28 sec.)	$2,69^{bcd}$	$18,97^{abc}$	$16,02^{abc}$
4	75 mM NaCl+90 W (56 sec.)	$0,89^{cd}$	$7,84^{bc}$	$9,56^{abc}$
5	150 mM NaCl	$4,27^{bc}$	$24,15^{ab}$	$5,87^{bc}$
6	150 mM NaCl+90 W (28 sec.)	$5,01^{b}$	$27,18^{a}$	$5,40^{bc}$
7	150 mM NaCl+90 W (56 sec.)	$1,06^{cd}$	$7,26^{bc}$	$6,88^{bc}$
8	90 W (28 sec.)	$0,85^{cd}$	$17,08^{abc}$	$20,09^{ab}$
9	90 W (56 sec.)	$0,23^{d}$	$5,13^{c}$	$22,30^{a}$

**The values that are followed by the same letter do not differ statistically at a significance level of 1% (P<0.01)*

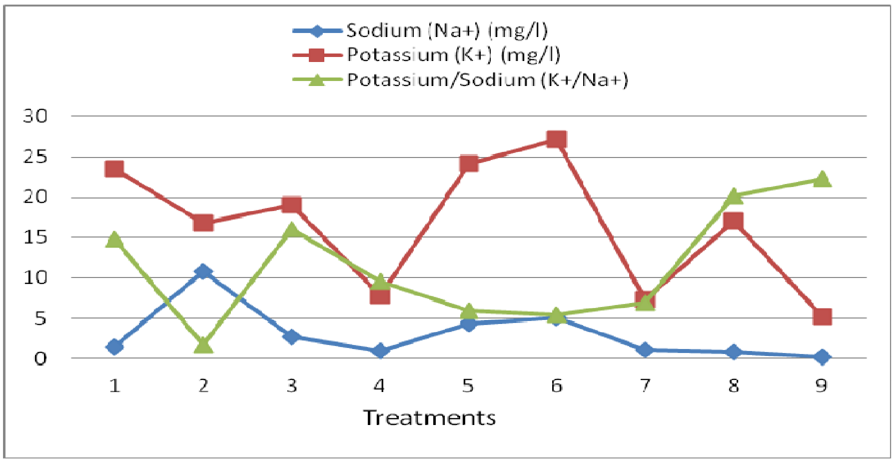


Figure 5. A diagram showing the effect of applications on Na+ and K+ amount and K+/Na+ ratio of plant tissue

The K^+/Na^+ ratio was found to be 14,75 for the control group plants, this rate was found to have decreased down to 1.65 in 75 mM with saline treatment, and to 5.87 in 150 mM (P<0.01) (Table 2). The K^+/Na^+ ratio was found to have increased with 75 mM NaCl+90W (28 sec. and 56 sn) treatment (P<0.01) (Fig. 5). In 150 mM NaCl+90 W (28 sec.) treatment, this score was found to have decreased with the single saline treatment, but it increased with 56 sec. treatment (Fig. 5). However, this increase and decrease were not found to be significant. The 90 W (28 sec. and 56 sec.) K^+/Na^+ ratio in the individual microwave treatment was found to be higher as 20,09 and 22,30 values compared to that of control group, which is 14,75 (P<0.01) (Table 2).

DISCUSSION

Salinity has many effects on plants growth and therefore, the criteria used in the choice of the plants resistant to salinity need to be discussed. The following features, such as root and shoot height, root/shoot ratio, germination rate, the rate of live and dead leaves, chlorophyll, carotenoid and proline amount are usually used in the choice of the plants. Some of these features were examined in corn [11], sweet corn [12], rice [13] and wheat [14] species. Besides, the microwave stress which is one of the other stress types, was also investigated with regards to its possible effects [7, 15].

Many researchers have revealed that increasing salinity prevents plant germination [16, 17, 18], represses the growth of shoot and root [19, 20, 21]. In this study, it was seen that the seeds exposed to both NaCl and microwave stress show weaker germination performance compared to the control group, which was germinated with the use of normal distilled water. This is an expected and natural result. In addition to this, it was revealed that the germination and germination energy has gradually increased in the seeds of *Gleditschia triacanthos* and *Robinia pseudoacacia* depending on the application energy [22]. Bhaskara Reddy et al. [23] suggest that low dose microwave has increased the germination rate, but as the amount of exposure to microwave increases, the germination performance decreases, and the tissue damage increases. In 150 mM NaCl treatment, whereas the germination was found to be 83.33%, it was found to be 86.66% in 150 mM NaCl+90W (28 sec.) treatment with a 4% increase ($P<0.01$). Similarly, the rate of germination which is 80% in 225 mM NaCl treatment, has increased to 83.33% in 225 mM NaCl+90W (28 sec.) treatment with a 4.16% increase ($P<0.01$) (Table 1). These results can be considered as evidences to suggest that weak microwave stress can improve the harmful effects of salinity stress even it is in low amount, or it can be considered that both stresses affect one another in a dual acting way.

Another result which can be suggested to be a strong evidence for this is that the germination rate in 90W (28sec.) has increased to 83.33% in 75 mM NaCl+ 90W (28 sec.) and increased to 86.66% in 150 mM NaCl+90 W (28 sec.) treatment ($P<0.01$). Whereas the germination rate was found to be 36.66% in 90W (56 sec.), the germination rate has increased with saline treatments and it reached up to 53.33% in 75 mM NaCl+90 W (56 sec.) treatment and reached to 60% in 50 mM NaCl+90 W (28 sec.) and 225 mM NaCl+ 90 W (28 sec.) treatment ($P<0.01$) (Table 1).

As the plant roots under salinity stress has significantly lost their ability to absorb water, their root and shoot growth start to regress. As the shoot diametre of the plants under stress decreases, accordingly, their heights are also affected by that. Jaleel et al. [24] suggest that salinity can inhibit plants by changing the water potentials, increasing ion toxicity or leading to ion unbalance. The decreases in the plant growth under NaCI stress have been suggested by many researchers [25, 26]. Sotiropoulos and Dimassi [27] have suggested that NaCl in low concentrations can increase the shoot height in vitro conditions. Flowers and Lauchli [28] have suggested that the positive effect of NaCl on plant growth may be as a result of osmolarity.

This study suggests that the salinity and microwave treatments increase the root lengths and shoot heights of the plants compared to the control group (0 mM NaCl) (Table 1). One of the results which we can present as a strong support to our hypothesis is that the root length in 75 mM NaCl treatment was found to be 3.20 cm and shoot height was found to be 4.90 cm. The 75 mM NaCl+90W (28 sec.) treatment has increased the root length to 6.10 cm, and shoot height to 5.55 cm.($P<0.01$) (Table 1).

The shoot height in 150 mM NaCl treatment was found to be 1.85 cm. The 150 mM NaCl+90W (28 sec.) treatment has increased the shoot height to 2.70 cm with a 45.94% increase. However, this increase was not found to be significant (Table 1).

In 225 mM NaCl treatment, 28 sec. microwave has increased the root length which is .95 cm to 3.80 cm, and the shoot height to 1.20 cm (Table 1) ($P<0.01$). 56 sec. microwave treatment was also found to have increased the root length ($P<0.01$). These are some of the other reasons why we think that microwave stress negatively affects salinity stress.

The individual microwave treatment was found to have increased the root lengths and shoot heights of plants compared to the control group. Whereas the root length was 4.40 cm in 0 mM NaCl control group and the shoot height was 6.25 cm, the root length was found to be 9.60 cm in 90 W (28 sec.) treatment and the shoot height was found to have increased to 7.0 cm ($P<0.01$). 56 sec. microwave treatment was also found to have significantly increased the root length. In other words, the individual microwave stress was found to have decreased the rate of seed germination, but some treatments were found to have increased the root length and shoot height. This is another interesting finding of this study.

The researchers suggest that it is an expected result that the root/shoot ratio increases in parallel with the increase in salinity density [29, 30], and that salinity affects plant growth more than root growth [31, 32]. Under the light of this data, the root/shoot ratio which decreased in 75 mM NaCl+90 W (56 sec.) (0.5353) and 150 mM NaCl+90 W (28 sec.) (0.5185) treatment compared to the control group, was found to have increased in all other treatments.

The studies carried out in the relevant field suggest that Na^+ has a antagonistic effect on K^+ and that the plants which can export Na^+ and replace K^+ are considered to be more resistant to salinity [33, 34] . Stavarek and Rains [35] suggest that the resistance of plants to salinity is dependent on their ability to mobilize the energy needed to export Na^+ and import K^+. In this study, this fact is strongly supported by the Na^+ amount which significantly decreased K^+ amount and which significantly increased in 75 mM NaCl+90 W (28 sec.) and 150 mM NaCl+90 W (56 sec.) treatments. Besides, the fact that Na^+ is significantly decreased in 90 W (28 and 56 sec.) treatments is also very interesting. These findings seem to confirm our conclusions suggesting that microwave treatments increases the tolerance to salinity.

The studies reveal that the fact that genotypes absorb different rates of Na^+ and K^+ and therefore, they have K^+/Na^+ in different rates (Na^+- K^+ division character) seems to be related to resistance to salinity [36, 37, 38]. Besides, Binsel et al. [39] points out that membrane function is affected not only by Na^+ and K^+ concentrations but also reduced K^+/Na^+ ratio. The fact that the microwave treatments (90 W 28 and 56 sec.) given as well as 75 mM NaCl significantly increases K^+/Na^+ ratio and that K^+/Na^+ ratio is quite high in individual microwave treatments are considered among the interesting findings of this study.

When we consider all the findings stated above, especially the microwave treatments for shorter periods (28 sec.) was found to be more effective in improving the negative effects of salinity stress compared to the treatments for longer periods (56 sec.) The same is true for salt concentrations. The germination rates of the 7-day plants in 75 and 150 mM NaCl concentrations, root length, shoot height, root/shoot ratio and K^+/Na^+ ratio microwave treatments have shown better improving performances.

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