

Full Length Research Paper

The influence of NaCl salinity on some vegetative and chemical changes of strawberries (*Fragaria x ananassa* L.)

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In this study, 500, 1000 and 1500 mg l⁻¹ salt applications in Kabarla and Gloria cultivars of strawberry in solid media culture were performed along with Hoagland nutrient solution. In the experiment, some vegetative growth and chemical changes were determined in the plants to which salt (NaCl) was applied compared with the control. At the end of the experiment, it was observed that Kabarla cultivar grew better under saline conditions compared to Gloria. It was observed that vegetative growth was generally restricted, depending on the increase in salt applications. Especially, damages on leaves became significant. It was determined that the increased salt doses affected chlorophyll and malondialdehyde levels, but these values were not sufficient to determine salt-tolerant cultivars. It was found that Na⁺ ion accumulation on root, crown and leaves of the plant and ion accumulation ratios of K⁺/Na⁺ and Ca²⁺/Na⁺ had significant effects on tolerance to saline conditions. Higher Na⁺ ion accumulation and ion accumulation ratios of K⁺/Na⁺ and Ca²⁺/Na⁺ were obtained in case of Kabarla, which was understood to grow better under saline conditions. These values stayed lower in case of Gloria.

Key words: Strawberry, NaCl salinity, vegetative, chemical.

INTRODUCTION

It is known that salt accumulation in soil and salt concentration of irrigation water encourage stain formation on leaves, weak vegetative growth and weak fruit production in strawberries (Kepenek and Koyuncu, 2002; D'Anna et al., 2003; Keutgen and Keutgen, 2003; Saied, 2005). Furthermore, it is known that they have forcing effects on stolon production (Ondrasek et al., 2006).

In the classification, which is done by considering growth levels of the plants under saline conditions, it was seen that strawberry takes place among most sensitive plant species (Maas and Hoffman, 1977; Bould et al., 1983). However, it was observed that strawberry cultivars were not affected at identical levels in case of identical salt concentrations (Saied et al., 2003; Casierra-Pasada and Garcia, 2005; Turhan and Eris, 2005). Besides genetic characteristics of strawberry cultivars (Dziadczyk et al.,

2003), type of the salt and certain conditions in root section (Martinez Barroso and Alvarez, 1997; Kaya et al., 2003) have effects on damage threshold. Strawberry plant is sensitive to Na⁺ and Cl⁻ ions accumulation and may be seriously damaged by them. It is expressed that Na⁺ and Cl⁻ ions accumulation in leaves are the most important factors affecting formation of salt damages (Hoffman, 1981; Martinez Barroso and Alvarez, 1997). It was determined that the cultivars having more tolerance to salt accumulated Cl⁻ ions in their roots and prevented the negative effect on the plant as a whole (Saied et al., 2003). However, it has been reported that the ratios of K⁺/Na⁺ and Ca²⁺/Na⁺ also affect salt tolerance of the plant (Yasar, 2007; Yıldız et al., 2008). As a result of salinity, K⁺ and Ca²⁺ accumulation decreases while Na content increases (Ruiz et al., 1999). In salt-tolerant strawberry cultivars, the ratios of K⁺/Na⁺ and Ca²⁺/Na⁺ are higher than those of salt-sensitive cultivars (Yıldız et al., 2008).

The plants under salt stress close their stomata to prevent water loss and therefore, prevent assimilation. This situation causes increase in number of free radicals and consequently, they decompose protein membrane lipids and

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nucleic acids as well as cell components such as chlorophyll (Makale et al., 1999). Degradation in cell membranes causes degradation in membrane permeability and selectivity. This situation causes increase in some elements while decrease in others inside the cell (Turhan and Eriş, 2004). Lipid peroxidation causes degradation of cell membranes and malondialdehyde (MDA) is generated as a product. Upon breakdown of chlorophyll, general appearance of the plant becomes yellow (Wise and Naylor, 1987).

In this study, the responses of the strawberry plants under salt stress were determined by employing day-neutral (Kabarla) and short-day (Gloria) strawberry cultivars. In both plants, besides the effects of applications at various salt doses on some vegetative characteristics, chlorophyll, malondialdehyde, Na^+ , K^+ and Ca^{2+} amounts were determined. These were determined in roots, crowns and leaves separately to determine in which section ion (Na^+ , K^+ , Ca^{2+}) accumulations were intensified. The ratios of K^+/Na^+ and $\text{Ca}^{2+}/\text{Na}^+$ in roots, crowns and leaves were taken into consideration.

MATERIALS AND METHODS

Plant materials and experimental conditions

In the experiment, frigo plants of Kabarla and Gloria cultivars were employed. The study was carried out in Plants Physiology Laboratories, Horticulture Department, Agricultural Faculty, Yüzüncü Yıl University.

The experiment was executed in the climatic room, where temperature and lightening period may be set up. The climatic room was arranged in a way that day/night temperature was 22/18°C, day length was 16 h and humidity was 65-70%. A cold white fluorescent lamp with $280 \mu\text{mol. m}^{-2}\text{.s}^{-1}$ was employed as light source. Both plants were planted in pots containing 1 liter sterile pumice. The plants were fed by adding Hoagland nutrient solution to the pots and it was supplied when required. Flower buds, which develop at early stages of plant's growth, were not allowed to grow and they were cut off. At the initial stage, salt was not added to nutrient solution and salt applications were started once the plantlets had had identical conditions after 30-day growth period. After this period, the plantlets had 4 or five leaves.

In the experiment, the salt concentrations of 500, 1000 and 1500 mg l^{-1} were applied. Salt (NaCl) was applied by adding it to Hoagland nutrient solution (Hoagland and Arnon 1950) and each time when the plants were supplied with the nutrient solution. In case of control plants, salt was not applied and they were supplied with normal Hoagland solution.

Fresh weight

Root, crown and leaves of each plant under analysis were measured separately. Weighing operations were performed by balances sensitive to 0.001 g.

Dry weight

The roots, crowns and leaves were placed in a drying cabin immediately after their fresh weights were determined. They were kept in drying cabin until their weights did not change anymore at

65°C and then, their weights were measured. Weighing operations were performed by balances sensitive to 0.001 g.

Surface areas of leaves

Developed leaves of each plant were counted and they were culled and then, their surface areas were measured in cm^2 by the help of the planometer.

Crown diameter

Change in thickness of the crown having significant effects on plant development was determined by calipers. Measurement was carried out on all plants in each application.

Chlorophyll

The three youngest leaves were taken and kept at -40°C in sealed glass jars until the analysis were performed. 200 mg of the frozen leaf samples at -40°C were taken and their absorbance values were read by a spectrophotometer at 654 nm after they left in a hot water bath, which was placed in 80% ethanol, at 80°C for 20 min (Luna et al., 2000). Total chlorophyll amount in fresh leaf samples were determined as mg.g^{-1} fresh weight.

Lipid peroxidation analysis

Malondialdehyde (MDA) amount as a product of lipid peroxidation, which can be called as damages in cell membrane and reported by Lutts et al. (1996), was determined. According to this method, 200 mg of leaf sample, which was prepared by the same processes found in chlorophyll analysis from plant sampling to storage in deep freezer, was taken and 5 ml of 0.1% trichloro acetic acid (TCA) was added to it. This mixture was centrifuged at 12500 rpm for 20 min and 3 ml supernatant was obtained from 5 ml of extract. 3 ml of 0.1% TCA containing 20% tio-barbituric acid (TBA) was added to it. The mixture was kept in hot water bath at 95°C for 30 min and then, their absorbance value was read by aspectrophotometer (Analytic Jena 40 model) at 532 and 600 nm. Total MDA amount in fresh leaf samples were determined as $\mu\text{mol.g}^{-1}$ fresh weight.

Ion analyses

The three youngest leaves were taken and kept at -40°C in sealed glass jars until the analysis were performed. 200 mg of the frozen leaf samples at -40°C were taken and placed in a beaker and 10 ml of 0.1 N HNO_3 (nitric acid) was added to it. Beakers were covered by aluminum foil and kept at room temperature for a week. After this period, the samples were shaken by the shaker for two hours and thus, they became ready for analysis. Na^+ , Ca^{2+} and K^+ ions were measured by flame photometric method (Eppendorf flame photometer). After measuring, ion amounts were determined as mg.g^{-1} fresh weight (Taleisnik et al., 1997).

Statistical analyses

The study was executed according to random trial pattern in 3 (three) replicates and 10 plants (10 pots) in each replicate. The results of the study were analysed according to Duncan's multiple comparison method. The letter was used in case the variances were not homogeneously distributed. The 5% probability level was accepted to indicate significances.

Table 1. Effect of NaCl applications on leaf areas, fresh leaf weights, fresh crown weights, fresh root weights, dried leaf weights, dried crown weights, dried root weights and crown sizes in Kabarla and Gloria strawberry cultivars.

Cultivar	Characteristic	Control	500 mg l ⁻¹	1000 mg l ⁻¹	1500 mg l ⁻¹
Kabarla	Leaf area (cm ² plant ⁻¹)	14.04 b ¹	15.57 a	15.18 a	5.24 c
	Fresh leaf weight (g plant ⁻¹)	2.78 b	4.042 a	3.285 ab	1.138 c
	Fresh crown weight (g plant ⁻¹)	3.22 c	4.420 b	5.913 a	5.623 a
	Fresh root weight (g plant ⁻¹)	6.30 b	6.594 b	7.287 ab	8.787 a
	Dried leaf weight (g plant ⁻¹)	0.63 ab	0.950 a	0.760 ab	0.247 b
	Dried crown weight (g plant ⁻¹)	0.75 b	0.955 ab	1.404 a	1.283 a
	Dried root weight (g plant ⁻¹)	1.25 b	1.461 a	1.375 ab	1.644 a
	Crown size (mm)	12.67 b	13.227 b	15.833 a	15.367 a
Gloria	Leaf area (cm ² plant ⁻¹)	15.933 a	15.733 a	13.333 b	12.167 b
	Fresh leaf weight (g plant ⁻¹)	0.863 a	0.670 a	0.437 b	0.257 b
	Fresh crown weight (g plant ⁻¹)	1.527 a	1.183 b	1.297 b	1.453 a
	Fresh root weight (g plant ⁻¹)	2.417 a	2.117 ab	1.980 b	2.090 b
	Dried leaf weight (g plant ⁻¹)	0.107 c	0.237 b	0.106 c	0.086 a
	Dried crown weight (g plant ⁻¹)	0.292 c	0.289 c	0.482 a	0.423 ab
	Dried root weight (g plant ⁻¹)	0.559 a	0.387 c	0.402 b	0.381 c
	Crown size (mm)	12.193 a	5.287 b	6.997 b	7.137 b

*Means followed by the same letter within cultivars and line are not significantly different at ($p < 0.05$) using Duncan's multiple range test.

RESULTS AND DISCUSSION

Vegetative characteristics

The effects of different salt applications on some characteristics (fresh leaf weight, leaf surface area, fresh root weight, crown diameter, crown number, dry leaf weight, dry root weight and dry crown weight) of Kabarla and Gloria strawberry cultivars are given in Table 1.

In this study, drying, which expands from edges of existing leaves through inside them, was observed in higher salt concentrations. This situation is an important indicator for damages from salt and also is employed to characterize excessive salt damage (Kepenek and Koyuncu, 2002; Casiera-Pasada and Garcia, 2005).

Furthermore, it was observed that new leaves did not develop sufficiently and stayed small in higher salt concentrations. A very significant decrease occurred in leaf surface area and accordingly fresh and dry leaf weights of Kabarla cultivar in 1500 mg l⁻¹ application. However, the response of Gloria cultivar was a slow decrease. In fact, it is expected that their responses will be different because of their genetic characteristics (Dziadczyk et al., 2003; Yıldız et al., 2008). Although a decrease in dry leaf weights was observed at higher doses, lower dose applications caused an increase in dry leaf weight. A significant increase is seen in 500 and 1000 mg l⁻¹ salt application for Kabarla cultivar and in 500 mg l⁻¹ application in Gloria cultivar. This situation was observed by Turhan and Eriş (2005) for Camarosa and Tioga cultivars also. When Turhan and Eriş (2005) increased salt application doses, dry leaf weight decreased

in Camarosa and Tioga.

In Kabarla cultivar, increased salt doses caused increases in fresh crown weight and accordingly dry crown weight. However, in Gloria cultivar, increased salt doses caused a very significant decrease in fresh crown weights and then, a slight increase in subsequent salt applications. However, it did not reach the levels of control plants. Considering dry crown weights in Gloria cultivar, contrary to fresh leaf weight, there is a general tendency to increase. This increase occurred at very higher levels in 1000 and 1500 mg l⁻¹ salt applications compared with control plant.

In Kabarla cultivar, it was observed that fresh and dry root weights increased depending on increase in salt applications. However, a slight decrease was seen in Gloria cultivar. Turhan and Eriş (2005) observed increase in Camarosa cultivar in 500 mg l⁻¹ salt application and decrease in subsequent salt application. The same researchers found a general decrease in Tioga cultivar although no variation was observed in 500 mg l⁻¹ salt application. In our study, variation in dry leaf weight of Kabarla cultivar develops in a quite different way from that of Camarosa and Tioga cultivars of Turhan and Eriş (2005).

It is known that strawberry plant is one of the most sensitive plants to salinity of soil and water. It has been observed in many researches that salt-borne damages caused very rapid variations in vegetative characteristics (Saied et al., 2003; Casiera-Pasada and Garcia, 2005). Considering variations in vegetative characteristics caused by salt applications, it was seen that Kabarla cultivar developed better than Gloria cultivar.

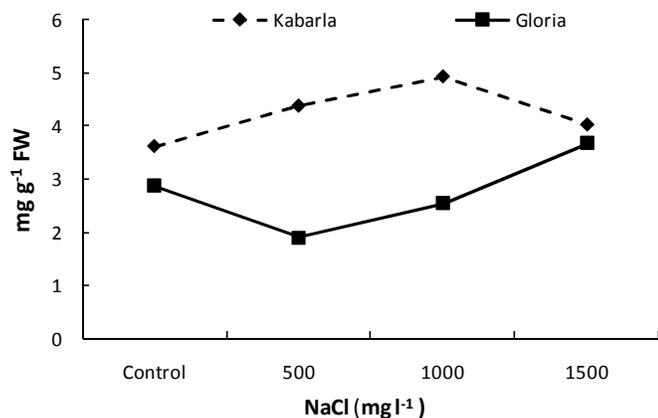


Figure 1. Effect of NaCl applications on chlorophyll (mg g⁻¹ FW) in Kabarla and Gloria strawberry cultivars.

Chemical characteristics

When the effects of salt applications on chlorophyll amount was investigated (Figure 1), it was seen that Kabarla and Gloria cultivars characteristics are in contrast to each other. Both cultivars contained similar amount of chlorophyll in control plants. An increase in chlorophyll amount was observed in Kabarla cultivar depending on salt applications. This increase reached the highest level in 1000 mg l⁻¹ application, followed by rapid decrease in 1500 mg l⁻¹ salt application. As seen in Figure 1, chlorophyll amount suddenly decreased in Gloria cultivar in 500 mg l⁻¹ salt application while it increased continuously in 1000 and 1500 mg l⁻¹ salt applications. It was found that chlorophyll level of both cultivars were quite similar in 1500 mg l⁻¹ salt application. Turhan and Eriş (2005) observed that the variation in chlorophyll amount caused by salt applications in Camarosa and Tioga cultivars is not important statistically. However, in our study, the variation in chlorophyll amount caused by salt applications both in Kabarla and also Gloria cultivars is important statistically. In a study performed by Kaya et al. (2002), it was found that salt application caused decrease in chlorophyll amount in the cultivars of Camarosa ve Oso Grande, which was important statistically.

Variation in malondialdehyde (MDA) accumulations, caused by cell membrane lipid peroxidation, depending on salt applications for Kabarla and Gloria cultivars are seen in Figure 2. Malondialdehyde increase is an expected event in plants under water stress (Wang, 1999). Salt applications cause negative effects on water intake of the plant. When the plant encounters water shortage problem, it will try to minimize water loss by closing its stomata. This situation inhibits photosynthesis and causes free radical formation inside the cell accordingly. Free radicals stimulate MDA formation as a product by causing lipid peroxidation in cell membrane beside other damages (Wang, 1999; Makale et al., 1999). As seen in

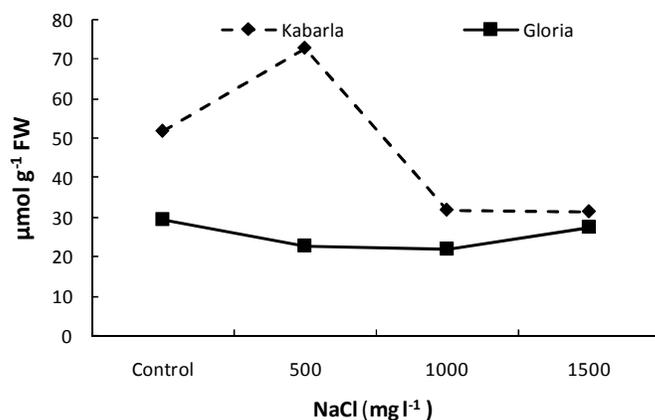


Figure 2. Effect of NaCl applications on malondialdehyde (MDA) (µmol g⁻¹ FW) in Kabarla and Gloria strawberry cultivars.

Figure 2, increasing salt applications did not affect MDA content in Kabarla and Gloria cultivars at the same level. In Gloria cultivar, contrary to what is expected, MDA tended to decrease initially, followed by increase at the highest dose. In Kabarla cultivar, although a quite high MDA content was observed compared with control plants at first dose, it decreased in subsequent salt applications. In a study by Yildiz et al. (2008), in all of 10 strawberry cultivars (Delmarvel, Douglas, Camarosa, Northester, Sweet Charlie, Tudla, Aiko, Elvira, Tioga, Rapella), it was seen that 100 mM NaCl application caused an increase in MDA amount compared to control plants. The reason for this variation may be that Yildiz et al. (2008) employed water culture in their study and we employed solid media culture.

The effects of salt applications on sodium (Na⁺), potassium (K⁺) and calcium (Ca²⁺) ion accumulation in root, crown and leaves are seen in Figure 3 for Kabarla and Gloria cultivars. The plants make selective ion intake under saline conditions. However, ion intake mechanism and physiological activities in plant organelles are seriously damaged under excessive saline conditions (Noble and Shannon, 1988; Wang, 1999; Saied et al., 2005). Calcium is an important element for smooth working of the metabolism of the plant (Cramer et al., 1986). Sodium ions have an important role in membrane permeability along with calcium ions. Therefore, it is claimed that high calcium levels protect cell membranes, against damages caused by excessive salt (Busch, 1995). Distribution of ion accumulations through organelles in strawberry plant is also one of the most important factors to determine level of the damages caused by salt. In this study, the most affected cultivar by salt is Gloria. Kabarla cultivar was considered more tolerant compared to Gloria. When sodium ion amounts in roots of both cultivars are taken into consideration, although decrease was seen in salt-sensitive Gloria cultivar, a continuous increase occurred in Kabarla. Considering potassium accumulations, any significant

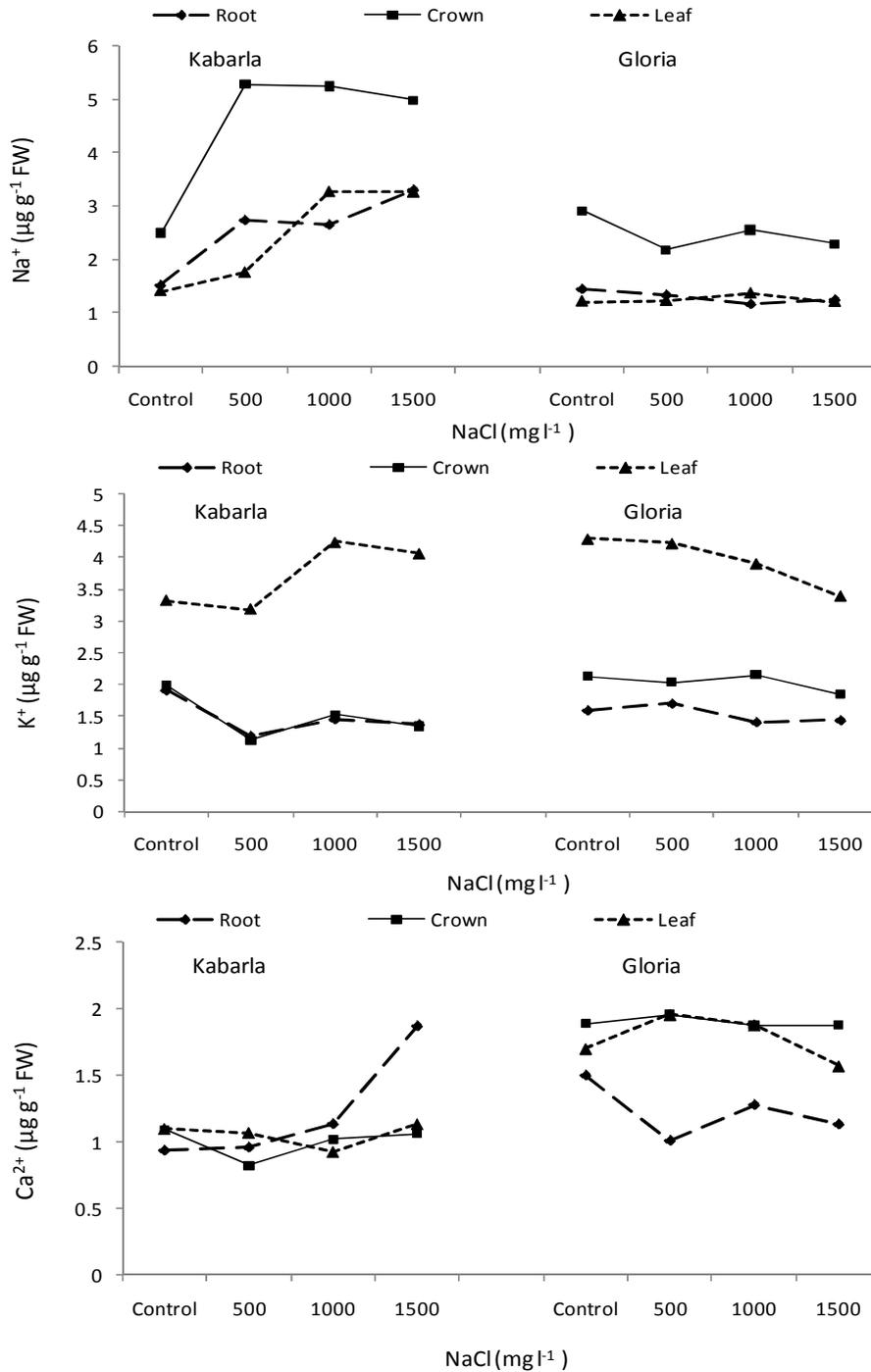


Figure 3. Effect of NaCl applications on sodium (Na⁺), potassium (K⁺) and calcium (Ca²⁺) ion accumulations (µg g⁻¹ FW) in root, crown and leaf of Kabarla and Gloria strawberry cultivars.

variation was not observed in Gloria cultivar. However, in Kabarla cultivar, a continuous decrease occurred in salt applications compared with the control. A continuous increase in calcium ion accumulations in the root of Kabarla cultivar, which is more salt-tolerant, was observed depending on the increase in salt applications.

However, in Gloria cultivar, a continuous decrease was observed. Calcium increase in the roots of salt-tolerant Kabarla gave similar results with evaluations of Busch (1995). Calcium accumulation, which increased in the root, caused the plant affected by salt lesser damages. This study suggests that potassium level in leaves also

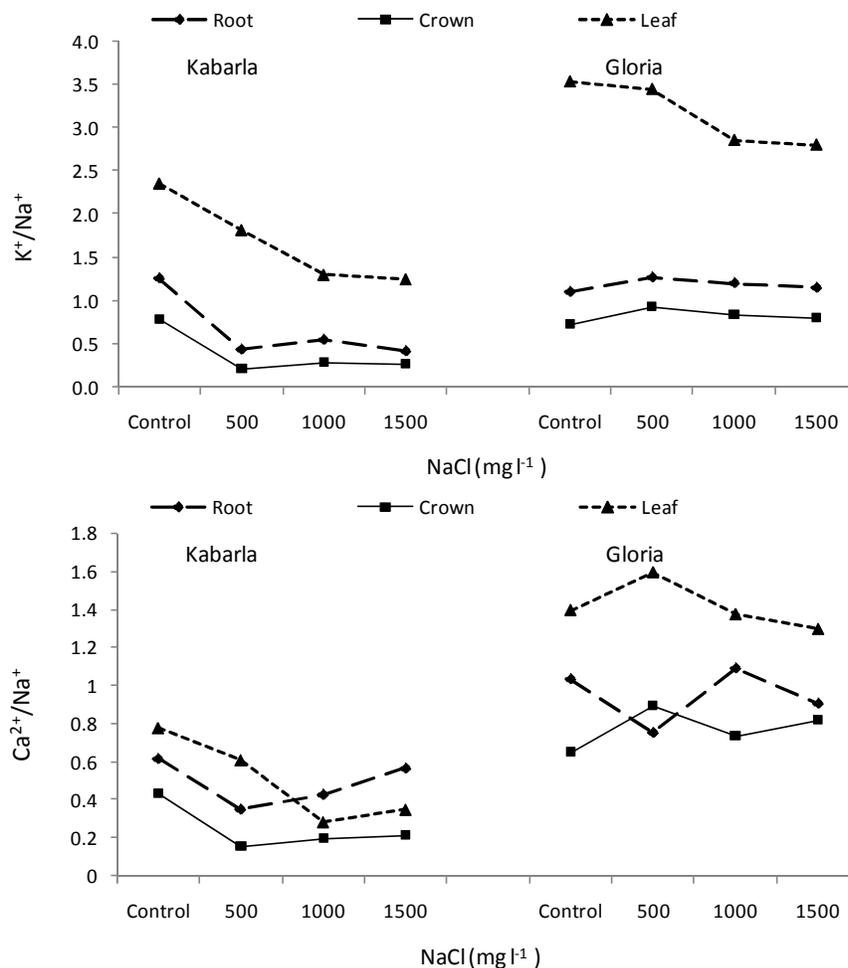


Figure 4. Effect of NaCl applications on K^+/Na^+ and Ca^{2+}/Na^+ ion accumulations rates in root, crown and leaf of Kabarla and Gloria strawberry cultivars.

has an indicative effect on salt-tolerance. It was found that in salt-sensitive Gloria cultivar, potassium ion accumulation decreased continuously with increase in salt applications. However, in case of Kabarla cultivar, it has a tendency to increase.

K^+/Na^+ and Ca^{2+}/Na^+ ratios can be considered as important indicators for salt tolerance (Yasar, 2007; Yildiz et al., 2008). The variation in K^+/Na^+ and Ca^{2+}/Na^+ ion accumulation ratios occurred depending on salt concentrations is seen in Figure 4 for Kabarla and Gloria cultivars. Yasar (2007) concluded that K^+/Na^+ and Ca^{2+}/Na^+ ratios are higher in salt-resistant genotypes of green peas compared with their sensitive genotypes. However, Yildiz et al. (2008) reported that this situation is not valid for strawberry plant. Yildiz et al. (2008) found that K^+/Na^+ and Ca^{2+}/Na^+ ratios are lower in tolerant cultivars of strawberry; however, these ratios are higher in sensitive cultivars. Our study supports the conclusions of Yildiz et al. (2008). Especially, considering K^+/Na^+ ion ratios, a significant different is seen between these two cultivars. The variations in K^+/Na^+ ion ratio, which is found

in root and crown as well as leaves, support this conclusion. It was found that Ca^{2+}/Na^+ ion ratios were lower in tolerant Kabarla cultivar when salt was applied compared to control plants. The decrease was seen in root, crown and leaves. In sensitive Gloria cultivar compared with Kabarla cultivar, although Ca^{2+}/Na^+ ratio has a tendency to increase in salt applications, this situation was not seen in all organelles at the same level. However, it is clear that although Ca^{2+}/Na^+ ion ratio did not increase in all organelles, any serious decrease did not occurred.

Although strawberry is among the most sensitive plant species, there are significant differences in sensitiveness between cultivars. In this study, it was determined that tolerance to saline conditions of Kabarla and Gloria cultivars was different significantly. It can be concluded that Na^+ ion accumulation in root and crown as well as leaves, K^+/Na^+ and Ca^{2+}/Na^+ ion accumulation ratios should be taken into consideration in tolerance of strawberry under saline conditions. In tolerant Kabarla, Na^+ ion accumulation, K^+/Na^+ and Ca^{2+}/Na^+ ion accumu-

lation ratios increases with salt applications. Contrary to that, in sensitive Gloria, these values decreased.

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