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Changes in Micronutrients, Chlorophyll Contents and Plant Growth of Some Strawberry Cultivars (*Fragaria x ananassa* L.) under Salt Stress

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Abstract: This study aimed to determine the effect of 2000 mg l^{-1} NaCl on micronutrients concentration, chlorophyll contents and plant growth in nine different strawberry cultivars. NaCl application did not cause a significant change in root and shoot weights. The leaf weight was decreased by salt treatment in all cultivars. This decrease was statistically significant in six cultivars (Carmine, CG-4, CG-2, CG-5, CG-3, Kabarla) and was not significant in three cultivars (Redland Hope, Sweet Charlie, and Camarosa). Total chlorophyll content was increased by NaCl treatment in all cultivars. While this increase was not statistically significant in some cultivars (Redland Hope, Sweet Charlie, CG-3, and Kabarla), increase in chlorophyll content was significant in other cultivars (Carmine, CG-4, CG-2, CG-5, and Camarosa). The changes in Mn, Zn, Fe, and Cu concentrations under saline conditions depended upon cultivars and the organs of plant.

Key Words: Chlorophyll, Micronutrients, Salt stress, Strawberry

Tuz Stresi Altındaki Bazı Çilek Çeşitlerinin Bitki Gelişimleri, Klorofil İçerikleri ve Mikro Besin Maddelerindeki Değişmeler

Özet: Bu çalışmada, dokuz farklı çilek çeşidinde bitki gelişimi, mikrobesin konsantrasyonu ve klorofil içerikleri üzerine 2000 mg l^{-1} NaCl'nin etkisini belirlemek amaçlanmıştır. NaCl uygulaması kök ve sürgün ağırlığı üzerine önemli bir değişime neden olmamıştır. Yaprak ağırlığı, tuz uygulaması ile tüm çeşitlerde azalmıştır. Bu azalma istatistiksel olarak altı çeşitte (Carmine, CG-4, CG-2, CG-5, CG-3 ve Kabarla) önemli iken üç çeşitte (Redland Hope, Sweet Charlie ve Camarosa) önemli bulunmamıştır. Toplam klorofil içeriği NaCl uygulamasıyla tüm çeşitlerde artmıştır. Bu artış bazı çeşitlerde istatistiksel olarak önemsizken (Redland Hope, Sweet Charlie, CG-3 ve Kabarla), diğer çeşitlerde (Carmine, CG-4, CG-2, CG-5 ve Camarosa) toplam klorofil içeriğinin artışı önemli bulunmuştur. Tuz uygulaması altında Mn, Zn, Fe, Cu konsantrasyonundaki değişmeler çeşitlere ve bitkinin organlarına bağlı olarak değişmiştir.

Anahtar kelimeler: Çilek, Klorofil, Mikrobesin maddeleri, Tuz stresi

Introduction

Salinity is a major abiotic environmental factor reducing plant growth and productivity throughout the world. About one-third of the world's irrigated land is affected by excess salinity (Hasegawa et al. 1986). In Turkey, 4 million ha of land are affected by salt and this area covers about 18% of the irrigable land (Sonmez 1990).

Salinity influences plant growth by its actions on osmotic pressure, on ionic toxicity due to excessive accumulation of one or several ions, and on the imbalance created between the ions. These factors have a greater or lesser degree of importance depending on the species under consideration and on the cultivar or varieties within the same species (Cornillon and Palloix 1997).

Most studies investigating a NaCl-induced nutritional disturbance have focused on excesses of Na^+ and Cl^- and when nutrients have been included, this has most often been limited to K and Ca. On the other hand, the studies on effect of salinity on micronutrient composition of plants were relatively in low number.

There are different ideas related to how salinity affects the micronutrient composition of plants. Hu and Schmidhalter (2001) suggested that the micronutrient concentration in plants is not much affected by salinity. However, Cornillon and Palloix (1997) reported that salinity increased the Zn concentrations in the root and leaf of pepper. A similar situation was stated for Cu and Mn in wheat and rice (Alpaslan et al. 1998). Villora et al. (2000) also indicated that concentrations of Fe, Mn, and Zn in leafs of zucchini were increased by increasing salinity, but concentrations of Cu was decreased. In a study conducted with two strawberries, Turhan and Eriş (2005) reported that with the salt applications, Fe and Mn content increased, while Cu content did not change in the aerial part of plants in two strawberry varieties.

In Turkey, strawberry production has rapidly increased in the last 20 years and has reached to 299 940 tons (Anonymous 2010). Salt stress causes serious problems in the strawberry cultivation at different level according to strawberry cultivars used. Therefore, this study aimed to determine the effect of 2000 mg l^{-1} NaCl on micronutrients and chlorophyll contents, and plant growth in nine different strawberry cultivars.

Material and Method

Nine strawberries cultivars ‘Redlands Hope’, ‘Sweet Charlie’, ‘Carmine’, ‘California 2’, ‘California 3’, ‘California 4’, ‘California 5’, ‘Kabarla’, and ‘Camarosa’ were grown in aquatic culture under 280 μmol m¯2 s¯1 of cool white fluorescent light of 16 photoperiod in a controlled climatic room at 22/18 °C day/night temperature, and 70 % relative humidity. Fifty cold-treated plantlets (frigo) of each cultivar were planted in plastic vessels filled with 5 L half-strength Hoagland’s solution (Hoagland and Arnon 1950). For each treatment, 30 plants similar in growth were finally used for the experiment. The solution in the vessels was replaced every week. Two weeks later, when the plants had developed four to five true leaves, salt-treatment started, and the NaCl concentration was increased by increments of 1000 mg l^{-1} day^{-1} until a final concentration of 2000 mg l^{-1} was achieved. Non-treated plants were kept as controls. Salt-stressed plants were subjected to 2000 mg l^{-1} NaCI for 30 d, and all plants, including controls, were sampled thereafter. The experiment was a completely randomized design with three replications per treatment, with ten plants each.

For micronutrient determination, dry samples of root, shoot and leaves were extracted in concentrated HNO_3 and $HCIO_4$. The Fe, Mn, Cu and Zn contents were determined by atomic absorption spectrometry (AAS).

For determination of chlorophyll contents, leaf segments (200 mg), frozen at –40°C, were placed in 5 ml of 80% ethanol and heated in a water bath at 80°C for 20 min. Total chlorophyll was evaluated in the alcohol extracts from absorbance readings, using the appropriate extinction coefficient. Chlorophyll content (mg g^{-1} fr wt) was calculated as 1000 x Ä654/(39.8 x sample fr wt) according to Tetley and Thimann (1974). All results reported were the means of three replicates. After data were analyzed by analyses of variance, the means were compared by use and Duncan’s multiple range test using SAS (1985) software.

Result and Discussion

The effects of NaCl applications on fresh weights of leaf root and shoot, and total chlorophyll contents of strawberry cultivars were shown in Table 1. As can be seen from the table, NaCI treatment did not cause significant differences in root weight for all cultivars. Similarly, shoot weight was not influenced by NaCI treatment in all cultivars with exception of CG-2 cultivars where salinity resulted in a pronounced decrease in shoot weight when compared to control treatments. The leaf weight was decreased by salt treatment in all cultivars. This decrease was statistically significant in six cultivars (Carmine, CG-4, CG-2, CG-5, CG-3, Kabarla), not significant in three cultivars (Redland Hope, Sweet Charlie, Camarosa). When taken into consideration the decreases in leaf weight, it may be suggested that Camarosa was the most tolerant one of the cultivars examined in this study. It was followed by cultivars Redland Hope and Sweet Charlie. In acccordance with the earlier studies (Martinez Barroso and Alvarez 1997; Saied et al.

2005; Turhan and Eriş 2005; Yıldız et al 2008), the results of this study also showed that the response of strawberry plants to salinity dependent on cultivars.

Total chlorophyll content was increased by NaCl treatment in all cultivars. While this increase was not statistically significant in some cultivars (Redland Hope, Sweet Charlie, CG-3, and Kabarla), increase of chlorophyll content was significant in other cultivars (Carmine, CG-4, CG-2, CG-5, and Camarosa). There have been different reports about this subject in the literature. For instance, chlorophyll content decreased with NaCl applications in lime (El Hag and Sidahmed 1997), mulberry (Agastian, Kingsley et al. 2000), and grapevine (Singh et al. 2000). In the same way, Kaya et al. (2002) have suggested that salt applications decreased total chlorophyll contents in the "Camarosa" and "Osogrande" strawberry varieties. However, there has been knowledge on increases of chlorophyll content in salty conditions depending on salt levels (Graifenberg et al. 1995; Güneş et al. 1996; Romero-Aranda et al. 2001). On the other hand, Turhan and Eriş (2005) reported that salt applications did not change total chlorophyll content in strawberry cvs Camarosa and Tiago.

Table 1. Fresh mass and chlorophyll contents of control and NaCl-treated strawberry cultivars.

Cultivars	Treatment	Root weight (g)	Shoot weight (g)	Leaf weight (g)	Chlorophyll (mg/g)
Redland Hope	Control	6.944	2.928	1.290	1.041
	NaCI	6.077	3.370	0.991	1.830
Sweet Charlie	Control	6.051	2.553	2.576	1.074
	NaCI	5.518	1.816	1.977	1.224
Carmine	Control	7.253	3.331	2.083	1.282
	NaCI	9.171	3.177	0.942*	2.814*
CG-4	Control	7.357	4.679	2.630	0.864
	NaCI	8.489	4.660	1.229*	2.449*
CG-2	Control	7.191	5.369	2.966	1.552
	NaCI	6.927	2.531*	1.505*	2.504*
CG-5	Control	5.185	4.249	2.312	1.238
	NaCI	6.645	2.230	0.565*	2.588*
CG-3	Control	5.742	2.703	2.707	0.884
	NaCI	5.824	3.281	1.158*	1.576
Kabarla	Control	5.339	1.384	3.046	1.455
	NaCI	5.631	1.846	1.774*	1.698
Camarosa	Control	2.659	0.988	1.594	0.683
	NaCI	3.741	1.032	1.463	1.752*
$LSD_{(0.05)}$		3.07	2.39	0.76	0.84

*Increasing or decreasing according to control is significant ($p< 0.05$)

Table 2 shows the Zn and Cu content of the roots, shoot and leaf of the nine cultivars studied. For the root Zn contents, a significant difference between control and NaCl treatment. was not found in all cultivars with exception Redland Hope, where salt treatment decreased significantly Zn content. Similarly, salt treatment did not caused a significant change in shoot Zn content of all cultivars with exception Sweet Charlie, where Zn content was decreased significantly by NaCI treatment. In all cultivars used in this study, leaf Zn contents were unaffected by the salt treatment. The reports in literature demonstrated that the effect of salinity on Zn content in plant depended on the type of plant. Zn concentration has been found to be increased in shoots of salt-stressed barley (Hassan et al. 1970a), soybean, squash, tomato (Maas et al. 1972), and in the grain of rice (Verma and Neue 1984), but decreased in shoots of corn (Hassan et al. 1970b) and mesquite (Khattak and Jarrell 1989).

The effect of NaCl on Cu content in different part of strawberry plant changed according to cultivars. When compared to control, the salt treatment decreased significantly root Cu content in Redland Hope, Kabarla and Camarosa, while it increased root Cu content in Carmine and CG-5. Cu content in shoot was increased by the salt treatment in Redland Hope, Sweet Charlie and CG-3. Similarly, while Cu content of leaf was significantly increased by NaCl treatment in Redland Hope, CG-2 and CG-3, it did not showed significant differences between control and salt treatment in the other cultivars. Turhan and Eriş (2005) reported that copper content did not change with salt applications in aerial part of two strawberry cvs

Camarosa and Tiago. Based on available literature obtained from different plant, the influence of salinity on Cu accumulation is variable. Some researchers revealed that the uptake of Cu generally increased in crop plants subjected to salinity stress (Alam 1994; Achakzai 2010). However, most other researchers indicated that in saline and saline sodic soils, the solubility of Cu is particularly low, and plants grown in such soils often experience deficiency of Cu (Page et al. 1990; Izzo et al. 1991; Rahman et al. 1993).

When compared to control treatment, the application of NaCl to nutrient solution caused significant changes in Fe content of some cultivars. In respect to Fe content in root and shoot, the responses of strawberry plants to salt stress were different depending on cultivars. While NaCI increased Fe content of some cultivars, it decreased Fe content in root and shoot of the other some cultivars. Leaf Fe contents were decreased significantly by salinity in Redland Hope, Carmine, CG-5 and CG-3, but did not show a significant change in the other cultivars (Table 3). Turhan and Eriş (2005) also reported that 2000 mg/L of NaCl application increased Fe content in aerial parts of Tiago cvs strawberry, but had no effect on Camarosa.

Table 2. Zn and Cu content in different organs of control and NaCl-treated strawberry cultivars.

Cultivars	Treatment	Zn (ppm)			Cu (ppm)		
		Root	Shoot	Leaf	Root	Shoot	Leaf
Redland	Control	36.56	13.51	13.17	14.04	4.24	4.19
Hope	NaCl	18.36*	15.28	15.60	9.45*	8.94*	6.71*
Sweet	Control	17.34	25.42	17.04	7.41	2.61	3.21
Charlie	NaCl	29.68	18.49*	15.98	4.99	6.16*	3.87
Carmine	Control	25.94	15.01	18.11	8.47	3.41	5.17
	NaCl	32.76	11.18	14.37	12.06*	5.22	4.37
CG-4	Control	27.24	10.99	12.56	7.80	2.78	3.47
	NaCl	19.06	12.55	13.57	7.60	4.29	2.72
CG-2	Control	19.87	14.33	17.05	4.33	2.37	2.87
	NaCl	20.45	11.16	15.17	6.98	4.73	5.63*
CG-5	Control	22.14	12.65	12.45	8.04	3.87	2.56
	NaCl	28.43	18.85	14.94	12.16*	6.81	3.58
CG-3	Control	31.31	13.84	14.13	5.22	3.10	2.50
	NaCl	21.25	13.60	13.63	8.07	7.72*	4.81*
Kabarla	Control	21.61	12.41	19.39	9.79	3.73	2.75
	NaCl	22.16	12.57	21.32	5.67*	4.14	4.06
Camarosa	Control	24.83	14.37	14.53	11.80	3.82	2.76
	NaCl	26.93	13.29	14.55	8.02*	6.84	3.31
$LSD_{(0.05)}$		14.95	5.47	6.27	3,57	3.21	1.43

*Increasing or decreasing according to control is significant ($p<0.05$)

The same authors determined that Fe content did not change with the salt applications the root part of both cultivars. There have been different results in literature about the effect of salt stress on Fe uptake depending on plant species used. It has been reported that salinity increased the Fe concentration in the shoots of pea (Dahiya and Singh 1976), tomato, soybean (Maas et al. 1972), and in the grain of rice (Verma and Neue 1984), but decreased its concentration in the shoots of barley and corn (Hassan et al. 1970a,b).

The root Mn contents were significantly decreased by salinity stress in Redland Hope, Camarosa, and CG-3 cultivars. On the other hand, Mn contents in root of other cultivars did not show significant difference between control and NaCl treated plant. The similar state was observed in shoot Mn contents of cultivars. The salt stress caused a pronounced decrease in Mn contents in shoot of CG-3 and Camarosa, but not significant change in other cultivars. In respect to leaf Mn content, the contradictory results were taken from two cultivars. Namely, Mn content was increased in leaf of Kabarla cultivars by NaCl application, but decreased in leaf of CG-3 cultivars. On the other hand, the differences between control and salt application were not found significant for Mn content in leaf of other cultivars. Turhan and Eriş (2005) reported that NaCl application increased Mn content in aerial part of Camarosa and Tiago cultivars. However, In this study, when compared to control, a significant difference resulting from NaCI treatment was not found for Mn content in leaf of Camarosa cvs. Moreover, Mn content in soot of Camarosa cultivars was significantly decreased by NaCl treatment (Table 3).

Table 3. Fe and Mn contents in different organs of control and NaCl-treated strawberry cultivars

Cultivars	Treatments	Fe (ppm)			Mn (ppm)		
		Root	Shoot	Leaf	Root	Shoot	Leaf
Redland Hope	Control	1745.62	48.35	73.67	176.42	14.82	59.25
	NaCl	944.39*	28.51	42.61*	78.70*	15.45	54.17
Sweet Charlie	Control	649.05	74.15	27.32	31.07	22.48	78.88
	NaCl	1046.65*	130.70*	38.10	43.36	23.72	86.31
Carmine	Control	680.41	36.67	46.00	86.11	15.30	46.16
	NaCl	1363.57*	39.34	29.04*	93.33	16.69	42.28
CG-4	Control	1218.06	38.88	41.92	76.65	15.26	67.79
	NaCl	800.65*	70.69	36.80	68.64	15.81	60.85
CG-2	Control	917.361	76.98	31.78	62.72	17.99	71.99
	NaCl	1363.92*	24.86*	29.62	77.47	20.42	61.67
CG-5	Control	1054.88	57.93	53.40	68.31	19.07	42.94
	NaCl	1218.82	39.69	30.55*	67.13	14.31	37.98
CG-3	Control	1488.33	109.76	62.97	77.52	27.00	75.27
	NaCl	1107.12*	25.65*	23.54*	30.37*	10.10*	47.11*
Kabarla	Control	1105.24	133.38	43.02	86.71	25.36	33.79
	NaCl	1969.78*	75.92*	33.09	95.03	20.93	78.19*
Camarosa	Control	1590.22	77.58	61.21	107.99	30.85	88.16
	NaCl	1808.71	108.29*	50.69	94.06	21.35*	73.18
$LSD_{(0.05)}$		300.8	32.45	13.8	28.51	7.66	17.47

*Increasing or decreasing according to control is significant ($p<0.05$)

In conclusion, the effect of salt stress on micronutrient composition in different part of nine strawberry cultivars showed pronounced differences depending on cultivars. Depending on the type of plant, salinity and micronutrient concentration in nutrient solution, the reports in literature also demonstrated that the effect of salinity on the micronutrients in plant is complex. Also, different varieties of the same species may differ in uptake efficiency of micronutrients as well (Hassen et al.1970a; Marschner 1995; Hu and Schmidhalter 2001).

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