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Effect of salt stress on concentration of nitrogen and phosphorus in root and leaf of strawberry plant

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Abstract

In this study the effect of salt stress on the concentrations of nitrogen (N) and phosphorus (P) in the leaves and the roots of two strawberry (*Fragaria vesca* L.) cultivars (Camarosa and Sweet Charlie) was investigated on cold stored bare-rooted seedlings grown in buckets filled with coarse sand. The treatments consisting of no-NaCl control, 1760, 2400, and 3040 mg L^{-1} of NaCl in half-strength Hoagland nutrient solution were applied to the plants for six months. During the experiment, leaf and root sampling were performed two times with five months interval. Roots and leaves of the plants were analyzed for Na, Cl, N and P. Analysis of variance (ANOVA) procedures was performed in Three Factors Completely Randomized Design for plant analysis results. Additionally orthogonal comparison was applied to the significant salinity effects. Cultivar and sampling time affected N, P, Na and Cl concentrations of the roots significantly. Cultivar-sampling time and sampling time-salinity interactions were significant for the N, P and Na concentrations of the roots. Salinity solely affected Cl concentrations of the roots significantly. All the treatments affected the concentrations of P, Na and Cl of the leaves significantly. The N concentrations of the leaves were affected significantly by only sampling time. Cultivar-salinity and sampling time-salinity interactions were found significant in the leaf N concentrations of the plants. The results show that the cultivars probably have different strategies in arrangement of N and P composition under salinity.

Keywords: NaCl **s**alinity, *Fragaria vesca* L., Camaraso, Sweet Charlie, mineral nutrients.

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Introduction

The increasing urbanization has expedited the demand of out-of-season strawberry growing all over the world. Aforesaid production has been mainly realized in greenhouse conditions and provided a momentous economic contribution to the growers. However, the pressure on the limited sources of good quality irrigation water has promoted the usage of relatively low quality, mostly saline, irrigation water in the production. Additionally higher yield expectation increased the excessive fertilizer application. These phenomena increased the salinity hazard in the soils of the production areas.

An increase in soil salinity commonly results in a reduction of water intake of plants. Passive nutrient uptake of the plants is related to water intake and any decrease in water availability causes to a reduction in uptake of many plant nutrients. Additionally the imbalance in composition of saline soil solution can also cause to uptake of some ions in excessive amounts such as Cl, Na and Mg. An increase in the concentration of these ions either has a toxic effect directly to the plants or promotes imbalance in plant nutrient metabolism

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(Ghafoor et al., 2004). According to Orcutt and Nielsen (2000) the most interesting interaction between salinity and macronutrient uptake is that between salinity and N accumulation. Botella et al. (1997) reported that NaCl reduced the accumulation of nitrate and ammonium when provided together to the plant. On the other hand limitations of P can occur in glycophytes like strawberry exposed to high salinity (Gorham, 1992). According to Kalifa et al. (2000) crop tolerance to high NaCl concentrations is partly related to inorganic P availability. High NaCl caused reduced P uptake in several crop species. Both root uptake and translocation of P to shoots were depressed. All these processes result in lower crop yields. Strawberry species are very salt sensitive (Larson, 1994) and suffer reductions in growth, quality, and yields (Awang et al., 1993 a,b) at soil electrical conductivities (EC_e) of above 1 dS m^{-1} (Carter, 1981). The species however, differ in their salt tolerance (Awang et al., 1993 a,b). The objectives of this research were to evaluate the effect of increasing NaCl-induced salinity on the concentrations of N and P in strawberry. In particular how salinity interferes with N and P concentrations of root and leaf, and how these changes are related to time.

Material and Methods

Plant material and salinity treatments

Two strawberry cultivars, Camarosa and Sweet Charlie were grown in high plastic tunnel at 25 ± 10 °C, 75 ± 25% Rh and natural photoperiod during Fall 2004 and Spring 2005. Cold stored bare-rooted strawberry seedlings were planted in 5–L buckets filled with coarse sand (0.6–0.8 mm particle size) on September 30, 2004. Three plants were planted in each bucket.

The treatments consisted of control (no-NaCl) and 1760 mg L^{-1}, 2400 mg L^{-1}, 3040 mg L^{-1} of NaCl (which are equal to 2.75 dS m^{-1}, 3.75 dS m^{-1} and 4.75 dS m^{-1} salinity respectively) in half-strength Hoagland nutrient solution. Tap water used in the preparation of the treatments contains approximately 109.57 mg L^{-1} Na and 7.1 mg L^{-1} Cl. Salinity treatments were started 30 days after planting. The pots irrigated with an amount of NaCl-enriched half concentrated Hoagland solution accounting for a leaching factor of 20–25% (Hoagland and Arnon, 1950) for six months. Each treatment was replicated 3 times with 2 buckets, 3 plants per bucket, hence 6 buckets and 18 plants per replicate.

Sampling and chemical analyses

Leaf and root sampling were performed two times with five months interval in full bloom periods of the plants. For this purpose the first three buckets in the each replication were emptied on January 2, 2005 and the second three buckets were emptied on May 1, 2005. The plants were gently removed from the sand and partitioned into roots and leaves. The roots were selected by hand mechanically from the sand. For plant analysis, physiologically mature leaves of the same physiological age, free of damage or defects, were sampled. The samples were immediately transported to the laboratory in closed polyethylene bags and carefully washed with tap water, rinsed in deionized water, and dried in a forced-air oven at 70°C for 72 h in paper bags. Dried samples were ground in a stainless steel coffee grinder. The ground samples were wet digested in a mixture of nitric acid/perchloric acid (HNO_3/$HClO_4$) (4/1, v/v) solution (Westerman, 1990). Sodium contents in the digest were determined using flame photometry (Jenway PFP7, Staffordshire, UK), P by the vanadomolybdophosphoric method (Westerman, 1990). The total N content of the dried samples were analyzed by Kjeldahl digestion method (Westerman, 1990). For the N analysis, 0.25 g of the samples was wet digested in a heating digester (Velp Scientifica, DK20, Milano, Italy) and then distilled in a distillation unit (Velp Scientifica, UDK126A, Milano, Italy). Aliquots were titrated by 0.1 N HCl. The results were expressed as the % N in the dry matter. The Cl was extracted from 0.1 g of the ground sample with 10 ml of deionized water by shaking the mixture for 2 h. The Cl concentrations of the extracts were measured by a chloridmeter (Jenway PCLM3, Staffordshire, UK). The results were expressed as the % Cl in the dry matter (Brown and Jackson, 1955).

Analysis of variance (ANOVA) procedures were performed in Three Factors Completely Randomized Design for plant analysis results according to Little and Hills (1978). Additionally orthogonal comparison was applied to the significant salinity effects.

Results

Variance analyses results of the effects on the cultivars, sampling time and salinity on N, P, Na and Cl concentrations of the roots and the leaves were given in Table 1. Cultivar and sampling time affected N, P, Na and Cl concentrations of the roots significantly. Cultivar-sampling time and sampling time-salinity

interactions were significant for the N, P and Na concentrations of the roots. Salinity solely affected Cl concentrations of the roots significantly. All the treatments affected Cl concentration of the roots however the interactions between the treatments were nonsignificant.

Table 1. Variance analysis results of the effects of cultivar, sampling time and salinity on N, P, Na and Cl concentrations of the roots and the leaves

Treatments and interactions	Nutrients in the roots				Nutrients in the leaves			
	N	P	Na	Cl	N	P	Na	Cl
A-Cultivar	**	**	**	**	ns	**	*	**
B-Sampling Time	**	**	**	**	**	**	**	**
C-Salinity	ns	ns	ns	**	ns	**	**	**
AxB Interaction	*	**	**	ns	ns	**	ns	ns
AxC Interaction	ns	ns	ns	ns	**	ns	ns	ns
BxC Interaction	*	*	**	ns	*	**	ns	ns
AxBxC Interaction	ns	ns	ns	ns	ns	ns	ns	ns

* P ≤ 0.05, ** P ≤ 0.01, ns: nonsignificant

All the treatments affected the concentrations of P, Na and Cl of the leaves significantly. In other words, different levels of P, Na and Cl concentrations of the cultivars altered according to sampling time and salinity levels. The N concentrations of the leaves were affected by only sampling time. Cultivar-salinity and sampling time-salinity interactions were found significant in the leaf N concentrations of the plants.

Cultivar-sampling time and sampling time-salinity interactions were significant for the leaf P concentrations of the plants. None of the interactions between the treatments were significant for Na and Cl concentrations of the leaves. Interaction between the cultivar and sampling time for N, P and Na concentrations of the roots were seen in Table 2. Nitrogen and P concentrations of the cultivars decreased, and Na concentration increased with time.

Table 2. Interaction between cultivar and sampling time in respect to root N, P and Na concentrations

Cultivar	Root N (%)		Root P (%)		Root Na (%)	
	January	May	January	May	January	May
Camarosa	3.25*	1.71	0.33	0.06	0.31	0.39
Sweet Charlie	3.87	2.08	0.35	0.13	0.56	0.76

Interaction and orthogonal comparisons between the sampling times and the salinity levels for the root N, P and Na concentrations were given in Table 3 and Table 4 respectively. Root N and P concentrations of the cultivars decreased and root Na concentrations increased with time (Table 3). Compared to the control treatment, increasing salinity levels enhanced the N and P concentrations of the roots in the first sampling time. This effect was linear for the N concentration. However aforesaid relations were nonsignificant in the second sampling time. Salinity treatments increased the concentration of Na of the roots linearly. Nitrogen and P concentrations of the roots were affected in the first sampling time (Table 4).

Table 3. Interaction between sampling time and salinity in respect to root N, P and Na concentrations

Plant Nutrients (%)	Sampling Time	Salinity (dS m^{-1})			
		Control	2.75	3.75	4.75
N	January	3.41*	3.41	3.68	3.74
	May	1.93	1.87	1.91	1.87
P	January	0.31	0.34	0.34	0.37
	May	0.10	0.10	0.09	0.09
Na	January	0.15	0.31	0.54	0.76
	May	0.45	0.52	0.50	0.84

Table 4. Orthogonal comparison of sampling time-salinity interaction in respect to root N, P and Na concentrations

Sampling Time	Comparison	N	P	Na
January	Control vs. other Treatments	*	**	**
	Salinity-Linear	**	ns	**
	Salinity-Quadratic	ns	ns	ns
May	Control vs. other Treatments	ns	ns	**
	Salinity-Linear	ns	ns	**
	Salinity-Quadratic	ns	ns	**

* P ≤ 0.05, ** P ≤ 0.01, ns: nonsignificant

Interaction between the cultivar and the salinity for the leaf N concentration, and their orthogonal comparisons were given in Table 5 and Table 6 respectively. The salinity affected leaf N concentrations of the cultivars in different ways. The leaf N concentration of Camarosa was stable, but the leaf N concentration of Sweet Charlie increased (Table 5). Probably the difference between the cultivars made the main effects of the salinity on leaf N concentration nonsignificant (Table 1). Compared to the control treatment, salinity levels increased the leaf N concentrations of Sweet Charlie, additionally salinity treatments affected the leaf N concentrations of this cultivar quadraticly. In other words, the second salinity level (3.75 dS m^{-1}) increased the leaf N concentration compared to the first salinity level (2.75 dS m^{-1}), however the third salinity level (4.75 dS m^{-1}) decreased the leaf N concentration of Sweet Charlie compared to the second salinity level (3.75 dS m^{-1}) (Table 6).

Table 5. Interaction between cultivar and salinity in respect to leaf N concentrations (%)

Cultivar	Salinity (dS m^{-1})			
	Control	2.75	3.75	4.75
Camarosa	2.24*	2.26	2.18	2.27
Sweet Charlie	2.18	2.16	2.37	2.34

Table 6. Orthogonal comparison of cultivar-salinity interaction in respect to leaf N concentrations

Cultivar	Comparison	
Camarosa	Control vs. other Treatments	ns
	Salinity-Linear	ns
	Salinity-Quadratic	ns
Sweet Charlie	Control vs. other Treatments	*
	Salinity-Linear	**
	Salinity-Quadratic	*

* P ≤ 0.05, ** P ≤ 0.01, ns: nonsignificant

Interaction between the sampling time and the salinity for the leaf N and P concentrations, and their orthogonal comparisons were given in Table 7 and Table 8 respectively. In general, the leaf N concentration of the cultivars increased with time, the leaf P concentration of the cultivars decreased with time (Table 7). The effects of the salinity levels on the leaf N and P concentrations of the cultivars disappeared in the second sampling times (Table 8). Salinity treatments increased the leaf N and P concentrations linearly in the first sampling time (Table 8).

Table 7. Interaction between sampling time and salinity in respect to leaf N (%) and P concentrations (%)

Plant Nutrients (%)	Sampling Time	Salinity (dS m^{-1})			
		Control	2.75	3.75	4.75
N	January	2.13*	2.10	2.19	2.31
	May	2.30	2.33	2.37	2.30
P	January	0.40	0.41	0.44	0.48
	May	0.20	0.22	0.20	0.21

Table 8. Orthogonal comparison of sampling time-salinity interaction in respect to leaf N (%) and P concentrations (%)

Sampling Time	Comparison	N	P
January	Control vs. other Treatments	ns	**
	Salinity-Linear	**	**
	Salinity-Quadratic	ns	ns
May	Control vs. other Treatments	ns	ns
	Salinity-Linear	ns	ns
	Salinity-Quadratic	ns	ns

** P ≤ 0.01, ns: nonsignificant

Interaction between the cultivar and sampling time for the leaf P concentration were given in Table 9. The leaf P concentrations of the cultivars decreased with time.

Table 9. Interaction between cultivar and sampling time in respect to leaf P concentrations (%)

Cultivar	Sampling Time	
	January	May
Camarosa	0.39*	0.20
Sweet Charlie	0.47	0.21

The effects of salinity on leaf Na, leaf Cl and root Cl concentrations, and their orthogonal comparisons were given in Table 10 and Table 11 respectively. The salinity treatments increased the concentrations of the related plant nutrients linearly.

Table 10. Effect of salinity on root Cl and leaf Na and Cl concentrations

Plant Nutrients (%)	Salinity (dS m^{-1})			
	Control	2.75	3.75	4.75
Leaf Na	0.01*	0.02	0.04	0.06
Leaf Cl	0.42	0.54	0.73	0.84
Root Cl	0.24	0.32	0.38	0.53

Table 11. Orthogonal comparison of salinity effect on root Cl, leaf Na and Cl concentrations

	Comparison of the nutrients in the leaves		Comparison of the nutrients in the roots
Interaction	Na	Cl	Cl
Control vs. other Treatments	**	**	**
Salinity-Linear	**	**	**
Salinity-Quadratic	ns	ns	ns

** $P \leq 0.01$, ns: nonsignificant

Discussion

The results of the mineral analyses showed that the cultivars probably have different strategies in point of the organization of Na in plants. Compared to the Camarosa, the root Na concentrations of the Sweet Charlie were approx. 45% and 49% higher for the sampling times of the January and the May respectively (Table 2). But it has slightly lower leaf Na concentration than that of Camarosa (The data not shown). Probably Camarosa tended to limit the entry of Na into the roots while Sweet Charlie tended to prevent translocation of Na to the leaves. In other words, Sweet Charlie has low translocation potential or a feedback control, from demand by vegetative growth that regulates the uptake and translocation from root to aerial parts. According to Jacoby (1979), ions accumulate in the root or in the basal part of the shoot, from where return to the root system and excreted into the medium. The efficiency of these exclusion processes varies among soybean (Durand and Lacan 1994) and olive (Demiral, 2005) cultivars, and can be considered as one of the mechanisms for maintenance of low leaf-Na concentration in plants.

Salt-tolerant plants differ from salt-sensitive ones in having a low rate of Na and Cl transport to leaves (Munns, 2002). Ulrich et al. (1980) report Na toxicities with concentrations greater than 0.1% Na in strawberry. Although there were differences in the root and leaf concentrations of Na between the cultivars, the leaf Na content of the cultivars did not exceed 0.05% in the study. Hence, the cultivars showed similar response in this point.

Chloride should also be appreciated in determination of salinity resistance level of plants. This element is absorbed by cell membranes and translocated easily to the upper plant parts compared to Na. Therefore, its level is generally higher than that of Na in upper plant parts although it is a trace element (Marschner, 1995). In this study, the cultivars significantly differed in the concentrations of Cl in their tissues (Table 1). Compared to the Sweet Charlie, Cl concentrations of root and leaf of Camarosa were lower both in the January and in the May sampling (Figures 1,2). Hence Camarosa can be rated as more tolerant cultivar to Cl in soil solution or in irrigation water. According to Grieve et al. (2003), the salt tolerance of soybean is positively correlated with Cl exclusion from the leaves and salt-sensitive cultivars' leaves are severely injured due to Cl toxicity and contained 10–15 times more Cl than the tolerant Cl excluding varieties. As reported by Demiral et al. (2005), salt-sensitive barley cultivar Kaya accumulated more Cl in leaves than salt-tolerant barley cultivar Scarlet.

Strawberry plant is sensitive to high Cl contents in root area (Lieten, 1997). Leaf Cl contents higher than 0.5% causes necrosis in leaves and yield reduce for most of the strawberry cultivars (Ulrich et al., 1980). In the study, increase in duration of salinity led the cultivars to exceed this limit in the second sampling period. Lieten (1997) stated that leaf Cl content of cv. Elsanta does not exceed 0.5% and shows no any toxicity symptoms in leaves under salinity.

Compared to the Camarosa, the root N concentrations of Sweet Charlie were approx. 18% and 16% higher for the sampling times of the January and the May respectively (Table 2). This phenomenon probably deals with the higher tissue Cl concentrations of Sweet Charlie than that of Camarosa (Figures 1, 2). Inhibition of

NO_3^- translocation to the leaves by Cl^- would result in an accumulation of NO_3^- in the roots (Rubinigg et al., 2003). According to the authors there might be a negative effect of NaCl on the translocation of NO_3^- from root to the aerial parts of the plants. As reported by Köhler and Raschke (2000) the anion channels with similar permeability for both NO_3^- and Cl^- in xylem parencyhma cells play a significant role in the xylem loading of NO_3^- and Cl^-. In the presence of high Cl^- concentrations, root to aerial parts translocation of NO_3^- could therefore be decreased at the site of entrance into the xylem via competition for the same channel.

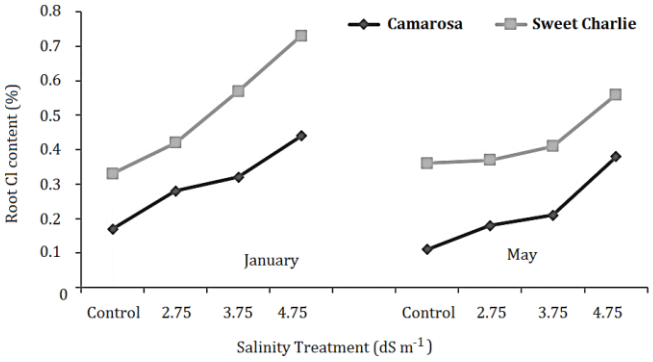

Figure 1. Variation in root Cl content of the cultivars under salinity in different sampling times

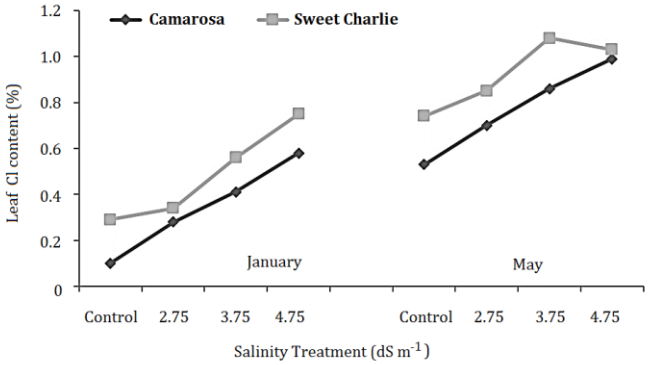

Figure 2. Variation in leaf Cl content of the cultivars under salinity in different sampling times

Another probable reason of high N concentration in plant tissues is increased synthesis of amino-N compounds as a plant response to salt stress. Probably increasing sensitivity of the plants to the salinity increased the synthesis of this kind of biochemical compounds in plants (Storey and Wyn-Jones, 1977). According to Loupassaki et al. (2002) salt sensitive olive cultivars have higher N concentration in their tissues. In the light of the data given above it can be concluded that Sweet Charlie is more salt sensitive cultivar than that of Camarosa and more salt sensitive plants have higher N concentrations in their roots than salt tolerant ones.

Salinity increased the N concentrations of the roots (Table 3). However this tendency disappeared in the second sampling time (Tables 3, 4). Compared to the sampling time of the January, the root N concentrations of the cultivars were approx. 47% lower in the sampling time of the May (Table 2). This might be a result of decreased NO_3^- influx under increasing Cl^- concentrations in plant tissues with time. According to Botella et al. (1994) Cl^- exerted a negative effect on NO_3^- uptake in *Triticum aestivum*, but could not specify whether the observed interaction was due to competitive inhibition or not.

The cultivars have lower concentrations of N in their leaves than that of their roots (Tables 2, 5). The lower N concentrations of the leaves of the plants could be the consequence of a generally lower rate of solute flow in the xylem as a result of a reduced transpiration rate for NO_3^- or amino acids, a lower requirement for NO_3^- in the aerial parts of the plants, or a decreased NO_3^- influx (Rubinigg et al., 2003). The concentrations of N of Sweet Charlie leaves significantly increased with the salinity (Table 5, 6). However this interaction was nonsignificant for Camarosa (Table 6). This result might be related to the different response of the cultivars to the salinity.

Compared to the sampling time of January, the N concentrations of the cultivars' leaves increased in the sampling time of May (Table 7). This might be related to the high amounts of the organic solutes synthesized under increased salinity stress resulted from increasing salinity level and salinity duration or a concentration effect due to growth depression. This tendency was significant in the January but in the May (Table 8).

The cultivars have more or less similar concentrations of P in their roots in the sampling time of January. However root P concentrations of the cultivars decreased significantly in the sampling time of May (Table 2). A similar tendency was also seen in the leaf P concentrations of the cultivars (Table 9).

Controversy, concerns about the effect of salinity on P concentrations of plants is reflected in the literature. Some researchers reported that salinity decreased P concentrations of plant tissues. According to Cangi and Tarakçıoğlu (2006) compared to control, 40 mM NaCl application decreased P in roots but increased in both leaves and shoots of kivifruit. Shibli et al. (2001) reported decreased of P in the shoots of *Saintpalia*

ionantha. Our results disagree with the aforementioned reports. On the other hand the results of some other studies indicated that salinity may increase P concentrations of the plants (Navarro et al., 2001; Loupassaki et al., 2002). Keutgen and Pawelzik (2009) found increased concentrations of P in roots and petioles of salt stressed strawberry cultivars Korona and Elsanta. Our results confirmed this increase of root (Tables 3) and leaf P concentrations in both cultivars in the sampling time of January but in the sampling time of May (Table 7). Sweet Charlie has both higher concentration of Cl (Figure 1, 2) and higher concentration of P in its tissues than that of Camarosa (Tables 2 and 9). Salinity imposed by Cl salts stimulated P uptake in plants (Cerda et al., 1977; Kasırğa and Demiral, 2016) and such increase of P levels is not a result of concentration effect due to growth depression (Roberts et al., 1984). According to Furihata et al. (1992) plants have two different P uptake systems: one with a high affinity (uptake of P at low P concentrations) and one with a lower affinity (uptake of P at higher P concentrations). The low affinity system is considered constitutive (Dunlop et al., 1997) and its activity is connected with the existence of multiple transporters of P in the plasma membrane and tonoplast (Schachtman et al., 1998). The transporters are regulated by the external P concentration (Leggewie et al., 1997) and high cytosol pH (Martinez and Lauchli, 1994). Most likely salinity increased the P uptake of the experimental plants through low affinity system and the salt-induced alkalinization of cytosols contributed to the process.

In conclusion; the results of the study showed that the cultivars and sampling times affected N, P, Na and Cl concentrations of the plants significantly. Under increasing salinity Camarosa suppressed better the concentrations of Na and Cl of the plant tissues than that of Sweet Charlie. Therefore Camarosa is rated as more tolerant cultivar to Na^+ and Cl^- ions in soil solution or in irrigation water. The effects of increasing salinity on plant nutrients tested have lost its significance over time. This is probably related to the increasing damage constituted by the extended stress to the plant metabolism. The cultivars showed different responses in terms of N and P contents under increasing salinity. Sweet Charlie had higher N concentrations in their tissues than that of the Camarosa. This reaction is evaluated as a sign of higher sensibility to salt stress. Salinity increased the P concentrations of the experimental plants and the cultivars had more or less similar concentrations of P in their tissues under salinity. This tendency is attributed to the changes in P uptake system of the plants that arising from the salinity.

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