

## Growth and Stomatal Behaviour of Two Strawberry Cultivars under Long-Term Salinity Stress

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**Abstract:** Sodium chloride (NaCl) treatments were conducted on strawberry plants (*Fragaria x ananassa* cvs. Camarosa and Chandler) grown under greenhouse conditions. Modified Hoagland solution (one-third strength) containing 0 (control), 8.5, 17.0 and 34.0 mM NaCl was applied to the plants for 6 months. High NaCl concentrations caused serious reductions in growth parameters such as fresh weight (FW) of leaves, stems and roots, leaf area and the number of leaves. Addition of salt to the growth medium caused a reduction in stomatal conductance (Gs) and transpiration rate (E) of Camarosa. Saline water up to 34 mM NaCl did not have any influence on Gs of Chandler. In addition, 34 mM NaCl treatment caused a marked increase in Gs and E of Chandler. On the other hand, leaf temperature (Ti) increased with salt treatments in both cultivars. We suggest that the reductions in stomatal conductance and transpiration rate represent adaptive mechanisms to cope with excessive salt in Camarosa. As it can relatively maintain its stomatal conductance and transpiration rate, Chandler also tolerates the salt injury at low salt concentrations. Considering the cultivars, Camarosa was characterised as more salt tolerant than Chandler under saline conditions.

**Key Words:** Strawberry, salt stress, plant growth, stomata

**Abbreviations:** Sodium chloride (NaCl), fresh weight (FW), stomatal conductance (Gs), transpiration rate (E), leaf temperature (Ti).

### Uzun Süreli Tuz Uygulamalarının İki Çilek Çeşidinde Büyüme ve Stoma Hareketlerine Etkisi

**Özet:** Sera koşullarında yetiştirilen çilek bitkilerine (*Fragaria x ananassa* cvs. Camarosa ve Chandler) NaCl uygulamaları yapılmıştır. Bitkiler 6 ay süre ile 0 (kontrol), 8.5, 17.0 ve 34.0 mM sodyum klorür (NaCl) içeren modifiye edilmiş 1/3 lük Hoagland besin çözeltisi ile sulanmışlardır. Tuzlu koşullar, yaprak, gövde ve kök yaş ağırlığı, yaprak alanı ve yaprak sayısı gibi büyüme parametrelerinde ciddi azalmalara neden olmuştur. Yetiştirme ortamlarına tuz ilave edilmesi Camarosa çeşidinde stoma iletkenliğini ve transpirasyon oranını düşürmüştür; Chandler çeşidinde ise, 34 mM a kadar tuz uygulaması stoma iletkenliğini etkilememiş; ancak 34 mM NaCl uygulaması stoma iletkenliğini ve transpirasyon oranını belirgin bir şekilde arttırmıştır. Yaprak sıcaklığının tuz uygulamaları ile birlikte her iki çeşitte de arttığı tespit edilmiştir. Camarosa çeşidinde stoma iletkenliği ve transpirasyon oranında görülen azalmanın tuz toleransta adaptasyon mekanizmasının bir parçası olduğu sonucuna varılmıştır. Düşük tuz konsantrasyonlarında stoma iletkenliği ve transpirasyon oranını nispeten koruyabilen Chandler çeşidinin de tuz zararını tolere edebildiği belirlenmiştir. Sonuç olarak, tuzlu koşullarda Camarosa'nın Chandler'a göre daha toleranslı olduğu belirlenmiştir.

**Anahtar Sözcükler:** Çilek, tuz stresi, bitki büyümesi, stoma

### Introduction

Increasing salinity of soil and water threatens agriculture and about one-third of the world's irrigated land is already affected by excess salinity (Hasegawa et al., 1986). In arid and semiarid regions of the world, limited rainfall, high evapotranspiration, high

temperature and inadequate water management each contribute to increases in soil salinity (Meloni et al., 2003). Therefore, plant response to salinity is one of the most widely researched subjects in plant physiology.

Salt stress with osmotic, nutritional and toxic effects prevents growth in many plant species (Hasegawa et al.,

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1986; Cheeseman, 1988). Therefore, the reduction in growth was explained by lower osmotic potential in the soil, which leads to decreased water uptake, reduced transpiration, and closure of stomata, which is associated with the reduced growth (Levitt, 1980; Ben-Asher et al., 2006). Plant species adjust to high salt concentrations by lowering tissue osmotic potential with the accumulation of inorganic ions (such as Na, K and Ca) as well as organic solutes (such as sugars, organic acids, free amino acids and proline) depending on species (Levitt, 1980; Hasegawa et al., 1986).

Strawberry cultivation (with 160,000 t production) (FAO, 2006) is of great importance in the horticulture sector in both domestic and foreign markets in Turkey. On the other hand, various dimensions of salt stress cause serious problems in strawberry cultivation. In strawberry, as well as in some other crops, the response of cultivars to salt stress has been well documented using agronomic and physiological characteristics (Dobren'Kova and Goncharova, 1986; Martinez-Barroso and Alvarez, 1997; Turhan, 2002; Turhan and Eris, 2004, 2005; Gulen et al., 2006). According to morphologic properties, ionic composition (Turhan, 2002), peroxidase profiles and electrolyte leakage (Gulen et al., 2006), the cultivars Camarosa and Tioga are more salt-tolerant than Chandler. Salt-induced injuries can occur due to the oxidative effects of salinity. Indeed, Gulen et al. (2006) have reported that strawberry plants must have a good antioxidative system or osmotic regulation in order to tolerate salt stress. It has been shown in our previous work that 8.5, 17.0 and 34.0 mM NaCl treatments for 10 weeks caused osmotic effects in the strawberry cultivar Camarosa and it has an osmotic regulatory ability under salt stress (Turhan and Eris, 2004).

Therefore, the aim of the present study was to investigate the morphologic and physiological changes in 2 strawberry cultivars induced by osmotic stress originating from long-term salt treatments and their role in salt tolerance.

## Materials and Methods

### Plant material and salt treatments

Seedlings of the strawberry cultivars Camarosa and Chandler were grown in perlite medium for 6 months. When the plants had developed 4-5 true leaves (20 days after transplanting), applications of modified Hoagland

solution (one-third strength) containing 0 (control), 8.5, 17.0 and 34.0 mM NaCl were started via drip irrigation. The composition of the nutrient solution was as follows: in g 1000 l<sup>-1</sup>; 38.32 monoammonium phosphate (MAP), 202.00 potassium nitrate (KNO<sub>3</sub>), 393.24 calcium nitrate [Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O], 164.00 magnesium sulphate (MgSO<sub>4</sub>·7H<sub>2</sub>O), 11.65 iron chelate (Fe-EDTA), 0.95 boric acid (H<sub>3</sub>BO<sub>3</sub>), 0.11 zinc sulphate (ZnSO<sub>4</sub>·7H<sub>2</sub>O), 0.0095 ammonium molybdate [(NH<sub>4</sub>)<sub>6</sub> Mo<sub>7</sub>O<sub>24</sub>·4H<sub>2</sub>O], 0.77 manganese sulphate (MnSO<sub>4</sub>·H<sub>2</sub>O), and 0.04 copper sulphate (CuSO<sub>4</sub>·5H<sub>2</sub>O). Plants were irrigated with their respective solution 4-6 times per day. It was attempted to keep the quantity of drainage water at 30% of the amount of nutrient solution applied. The electrical conductivity in the medium was 2.0 ± 0.2 (control), 3.0 ± 0.4, 3.8 ± 0.5 and 5.0 ± 0.6 dS m<sup>-1</sup>, respectively. The salt level was gradually increased over 1 week to avoid osmotic shock.

Plants were grown in a controlled greenhouse with day/night mean temperature of 25/10 °C, average relative humidity of 80%, and average photoperiod of 16 h. The experiment was set up using a randomised block design and replicated 3 times. There was 1 plant in each pot (1 l), with 10 pots in each replicate.

### Growth measurement

At the end of the experiment, plants were separated into leaf, stem and root parts and their fresh weights (FW) were directly determined. Number of leaves was also recorded. Total leaf area was measured using a planimeter (Placom KP-90N). For standardising data, the results were expressed as the relative reduction of yield on comparison to the control using the following formula:

$$\text{Relative reduction (\%)} = [(1 - (\text{salinised/control})) * 100$$

### Physiological measurement

During the experiment, stomatal conductance (Gs) and transpiration ratio (E) of leaves and leaf temperature (Tl) were determined using a portable steady state porometer (LI-1600M, LI-COR). Measurements were obtained every 3 weeks in the same leaves, during the middle of the photoperiod. The statistical evaluation took into consideration the average of the all measurements.

### Statistical analysis

The data were subjected to ANOVA and the means were compared by the least significant difference (LSD) at 0.05 confidence level using the BARNES and MSTAT-C computer programs, respectively.

## Results

### Effect of NaCl on growth parameters

Leaf, stem and root FW, number of leaves and leaf area were used to assess the adverse effect of high salinity on plant growth. Growth responses of strawberry plants to different salt concentrations in the medium are shown in Table 1. NaCl caused a significant suppression in the vegetative growth of both strawberry cultivars (Table 1, Figure 1). The reduction was greater at higher NaCl concentrations. FW gradually decreased with an increase in NaCl concentration for leaves and stems. The reductions were more pronounced at 17.0 and 34.0 mM NaCl, especially for Chandler. The root FW was also affected by NaCl treatment. The reduction was the highest in Chandler and reached 58.05% and 54.06% of the control at 17.0 and 34.0 mM, respectively. There was no reduction in leaf number in Camarosa at 8.5 mM NaCl. On the other hand, leaf number was reduced by 64.73% in Chandler at 34.0 mM NaCl treatment compared with

the control treatment. Compared to that of the control treatment leaf area was reduced by 77% in Camarosa and 71% in Chandler at 34.0 mM salt application.

### Effect of NaCl on physiological parameters

The effects of salinity on Gs and E of leaves, and Ti are shown in Figure 2. Addition of salt to the growth medium caused a reduction in Gs and E of Camarosa. Gs was reduced from 143.555  $\text{mmol m}^{-2}\text{s}^{-1}$  to 82.16  $\text{mmol m}^{-2}\text{s}^{-1}$  with the increase of NaCl concentration in Camarosa. E showed a similar trend (2.327  $\text{mmol m}^{-2}\text{s}^{-1}$  in the control treatment, 1.342  $\text{mmol m}^{-2}\text{s}^{-1}$  at 34 mM NaCl). However, saline water up to 34 mM NaCl did not have any influence on Gs of Chandler. In addition, 34 mM NaCl treatment caused a marked increase in Gs and E of Chandler. The highest Gs (101.37  $\text{mmol m}^{-2}\text{s}^{-1}$ ) and E (2.426  $\text{mmol m}^{-2}\text{s}^{-1}$ ) were observed with 34 mM NaCl treatment in Chandler. On the other hand, Ti increased gradually with the increase in NaCl concentration in Camarosa from 21.06 to 22.48 °C and in Chandler from 21.37 to 21.95 °C. In all the physiological parameters, the interaction between salinity and cultivars was significant as an indicator of the varietal difference to salinity.

Table 1. Effect of increasing NaCl concentration in the growth medium on growth parameters in plants of 2 strawberry cultivars, when plants were subjected for 6 months to NaCl treatments.

NaCl (mM)	Cultivar	Leaf FW (g)	Stem FW (g)	Root FW (g)	Number of leaves	Leaf area ( $\text{cm}^2$ )
0 (Control)	Camarosa	70.33 b	43.00 a	117.78	46.00	140.73
	Chandler	87.33 a	40.43 a	112.11	44.33	121.36
8.5	Camarosa	51.00 c	31.33 b	74.00	46.00	80.12
	Chandler	41.00 c	29.89 b	60.22	34.33	89.70
17.0	Camarosa	22.56 d	28.00 b	73.33	20.89	39.77
	Chandler	15.67 d	22.33 c	47.33	18.44	34.58
34.0	Camarosa	25.89 d	27.78 b	56.78	28.11	32.81
	Chandler	12.11 d	12.44 d	51.44	16.00	33.17
ANOVA						
NaCl (A)		ns	*	*	*	*
Cultivar (B)		*	*	*	*	ns
A*B		*	*	ns	ns	ns

ns and \* denote not significant and significant, respectively. Values not associated with the same letter are significantly different ( $P < 0.05$ ).

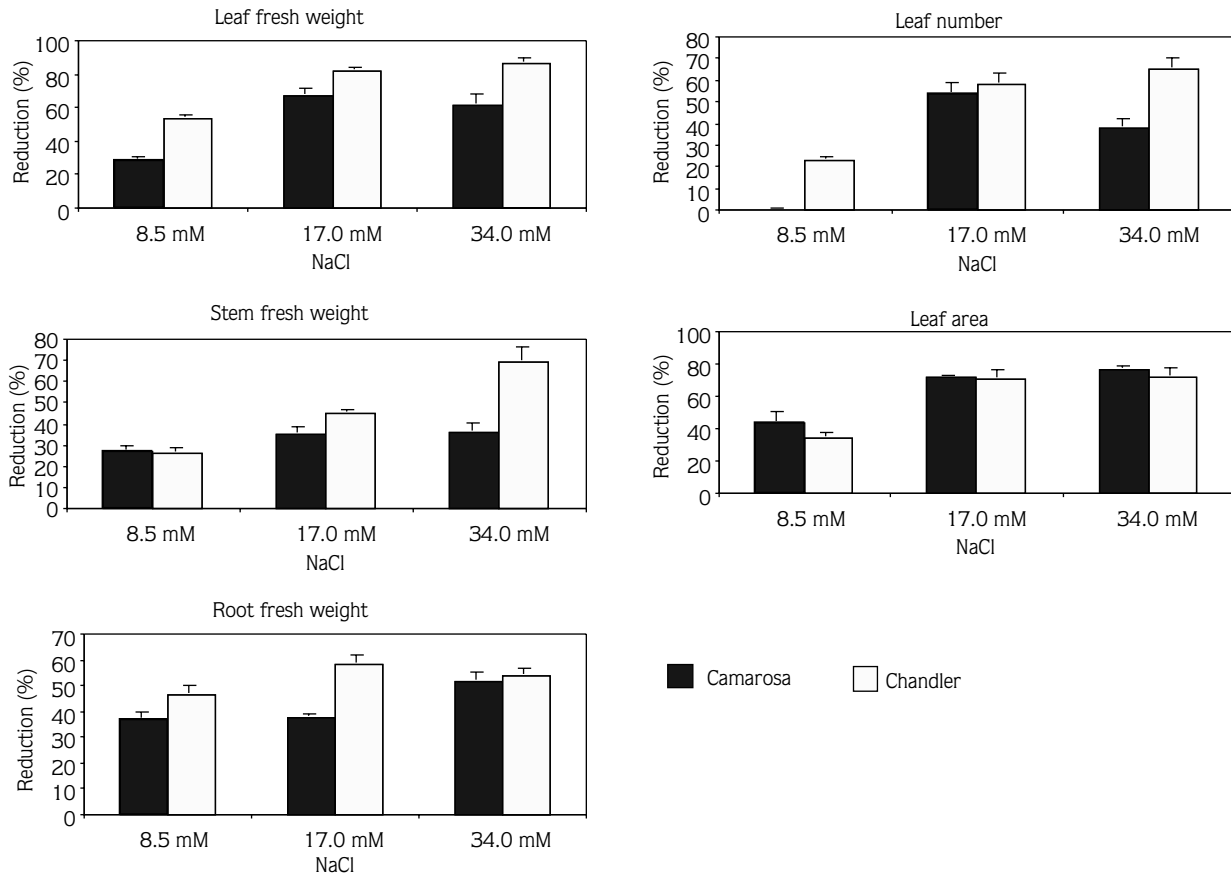


Figure 1. Effect of increasing NaCl concentration in the growth medium on growth parameters in plants of 2 strawberry cultivars. The plants were subjected for 6 months to NaCl treatments. Results are means of 3 replicates and expressed as reduction percentage of control. Error bars ( $\pm$  SE) are shown.  $n = 3$ .

## Discussion

Strawberry has been described by various authors as sensitive to salinity (Levitt, 1980; Schwarz, 1995). It is evident from the results that the long-term NaCl treatment caused a decrease in all of the growth parameters considered in both strawberry cultivars (Table 1, Figure 1). Thus, FW values of leaves, stems and roots were reduced by 17.0 and 34.0 mM NaCl. Salt stress also results in a considerable decrease in the FW of leaves and roots in sugar beet (Ghoulam et al., 2002) and the FW of shoots and roots in legumes (Ashraf and Bashir, 2003), in parallel with our results. Leaf number and leaf area also decreased with increasing salt concentration (Table 1, Figure 1). These findings are compatible with those reported by Dobren'Kova and Goncharova (1986), who studied strawberry. Similar results were reported for other species such as sugar beet

(Ghoulam et al., 2002) and legumes (Ashraf and Bashir, 2003). The decline in leaf growth is the earliest response of glycophytes exposed to salt stress. Growth inhibition in the long term is related to lower photosynthetic area (Munns and Termeat, 1986). However, decreases in leaf number were not only connected with the growth inhibiting effects of salt, but also to the injurious effects of salt due to defoliation of the damaged leaves. Salt-induced osmotic stress causes osmotic dehydration. This osmotic dehydration leads rapidly to a decrease in the osmotic and water potential of cells and in cell volume (Levitt, 1980). According to the present study, the growth reduction due to higher NaCl concentration can be attributed to the osmotic effect of salts. In fact, it was determined that salt treatments decrease leaf water potential and osmotic potential (Awang et al., 1993) and cause osmotic stress in strawberry (Turhan, 2002;

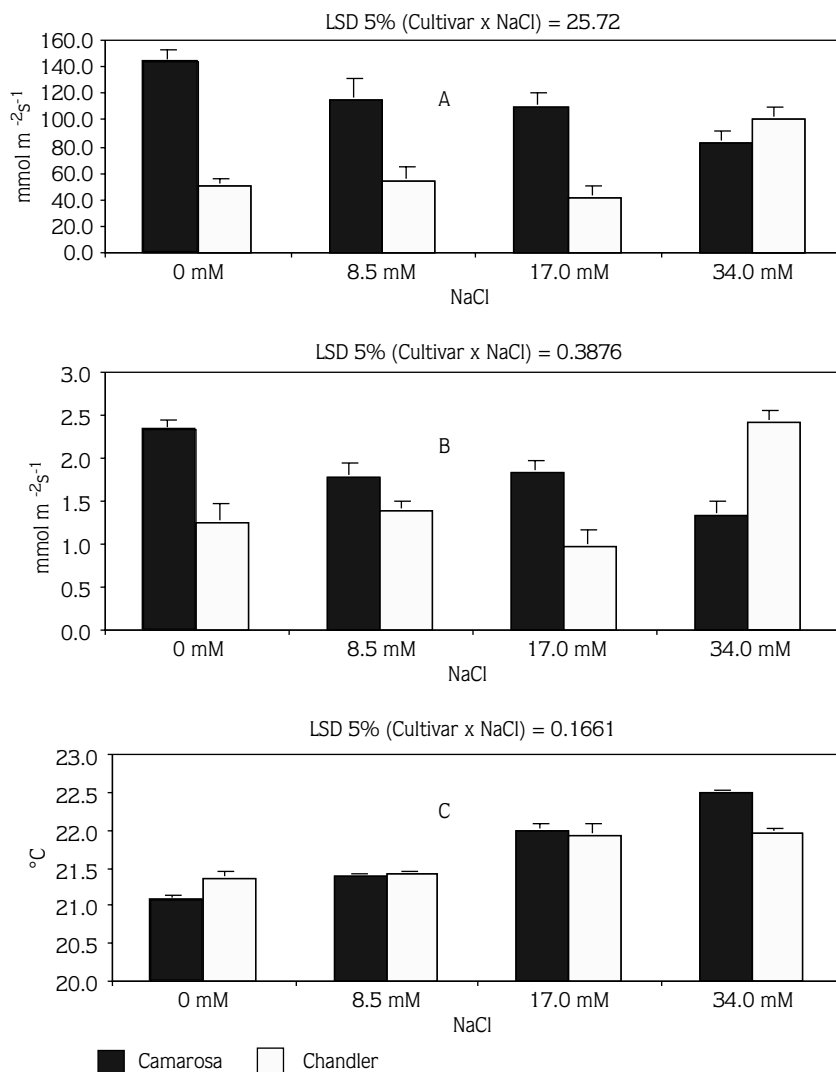


Figure 2. Effect of increasing NaCl concentration in the growth medium on stomatal conductance (Gs) (A), Transpiration rate (E) (B) of leaves and leaf temperature (Ti) (C) of 2 strawberry cultivars when plants were subjected for 6 months to NaCl treatments. Error bars ( $\pm$  SE) are shown.  $n = 3$ .

Turhan and Eris, 2004). The growth reduction induced by salinity has also been explained by a suppression of nutrient absorption due to uptake of NaCl in competition with nutrient ions (Levitt, 1980; Salisbury and Ross, 1992). As a matter of fact, Turhan (2002) and Turhan and Eris (2004) have concluded that with the effect of salt treatments the amounts of Na and Cl increased, while the amount of K decreased.

Specific toxic effects of salt also reduce plant growth. It was determined that NaCl treatments increased Cl

content in strawberry plants (Martinez-Barroso and Alvarez, 1997; Turhan, 2002; Turhan and Eris, 2004). According to Levitt (1980), NaCl treatments increase Na and Cl accumulation and toxic effects related to the accumulation of these ions cause necroses and moulding in leaves.

Addition of salt to the growth medium caused a reduction in Gs and E of Camarosa (Figure 2). Similar results were obtained in mulberry (Agastian et al., 2000), legumes (Ashraf and Bashir, 2003) and pepper plants

(Martinez-Ballesta et al., 2004; Lycoskoufis et al., 2005), where  $G_s$  and  $E$  were reduced by salt treatments. On the other hand, saline water (up to 34 mM) did not have any influence on  $G_s$  of Chandler. In addition, 34 mM NaCl treatment caused a marked increase in  $G_s$  and  $E$  of Chandler. A decrease in  $G_s$  was observed for the NaCl treatments, probably caused by closure of the stomata or a decrease in water uptake through the roots. The decrease in water flow due to salt stress may cause lowering in leaf water content that would result in stomatal closure in order to maintain their water status (Robinson et al., 1997). The quick response to NaCl observed in strawberry plants, in terms of water loss and a reduction in  $G_s$ , could indicate a certain level of adaptation of this crop to salt stress (Munns and Termaat, 1986). Similar results were obtained by Martinez-Ballesta et al. (2004) and Koyro (2006) for pepper and *Plantago coronopus*, respectively. It has been proposed that the reduction in leaf gas exchange in response to salinity is due to an increase in leaf Na concentration (Garcia-Legaz et al., 1993). However, other authors associated reductions in photosynthetic capacity and stomatal conductance with high concentrations of Cl (Banuls et al., 1997). Turhan (2002), working with the same strawberry cultivars, concluded that long-term salinity treatments increased Na and Cl content in strawberry plants. Therefore, in strawberry plants, an effect of both Na and Cl may have occurred.

Transpiration rates generally tend to decline with increasing rhizospheric salinity in both halophytes and non-halophytes. This might be due to lowered water potentials in the roots, and the transfer of abscisic acid from root to shoot as a signal, but at higher concentrations it could also be the result of inhibition of photosynthesis caused by salt accumulation in the mesophyll, and increasing intercellular  $CO_2$  concentrations, which reduce stomatal apertures (Robinson et al., 1997). On the other hand, salt treatments caused an increase in  $T_i$  in both strawberry cultivars in the present study (Figure 2). As evidence, in leaves that lose transpiration ability the temperature increases (Levitt, 1980).

In conclusion, the assessment of the effect of salinity on the growth parameters in 2 strawberry cultivars allows us to conclude that all of the considered parameters were affected by salinity with a varietal difference. Indeed, the cultivar Camarosa was more tolerant than Chandler. Considering the physiological parameters, we suggest that the reductions in stomatal conductance and transpiration rate represent adaptive mechanisms to cope with excessive salt, especially in Camarosa. On the other hand, Chandler also tolerates the salt injury at low salt concentrations because it can maintain its stomatal conductance and transpiration rate at low salt concentrations.

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