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RESPONSE OF SIX STRAWBERRY CULTIVARS TO SALINITY DURING THE *IN VITRO* PROLIFERATION AND ROOTING STAGES AND DURING POST-ACCLIMATIZATION PERIOD IN THE GREENHOUSE

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ABSTRACT: **Six strawberry cultivars: Camarosa, Carlsbad, Sweet Charlie, Seascape, Capitola and Chandler were tested for growth under different salinity levels (0.0 – 0.8% NaCl) in the tissue culture media during the *in vitro* proliferation and rooting stages followed by greenhouse testing for salt tolerance of the same cultivars (at 0.0 – 0.5% NaCl). Results of the *in vitro* experiments showed significant variations among strawberry cultivars for salt tolerance. Based on several growth parameters, including shoot fresh and dry weights, proliferation and rooting capacities, the cultivar Carlsbad showed higher salinity tolerance while Chandler was the most salt sensitive among the six tested cultivars. When tissues of these two cultivars were analyzed for nutrients and proline contents under salinity stress, Carlsbad was shown to accumulate more N, K, Ca and proline and less Na than Chandler, which confirmed its relative tolerance to salinity. Results also showed that increasing NaCl levels in the medium resulted in significant reduction in all growth parameters during *in vitro* proliferation and rooting stages. Salinity also decreased tissue N, K and Ca, but increased tissue Na and Mg as well as proline. Results of cultivar response to salinity under greenhouse conditions were almost similar to those under *in vitro* conditions. In addition, chlorophyll contents were higher in the leaves of Seascape and Carlsbad as compared to the other cultivars under the highest NaCl level. A significant reduction in chlorophyll was also noted with each increase in NaCl application rate. Under**

greenhouse condition, none of the cultivars survived NaCl levels higher than 0.3%, in contrast to those survived up to 0.8% NaCl during the *in vitro* experiments, indicating variations in plants response to salinity in relation to their developmental stage.

INTRODUCTION

Due to the increasing demand for growing strawberries for export, several cultivars of different origins have been introduced to Egypt through the past few years. The introduction of these modern cultivars was mainly based on their yield potentials, irrespective of their adaptability or sensitivity to stress conditions such as salinity. According to Maas and Hoffman (1977), the 50% yield threshold for salt tolerance in strawberry is an EC of 1.0 dSm^{-1}, which put the crop among the most sensitive vegetables to salinity.

Excessive salinity is a major limiting factor in crop production in the world (Tal, 1985). Leaching excess salts from soils is expensive and impractical. Therefore, the use of crop varieties that are tolerant to saline conditions is essential. However, field screening in saline sites is complicated due to the large variation in soil salinity levels and different ontogenic reactions of the plant to salinity. Testing micropropagated plantlets in *in vitro* saline media could overcome some of these *in vivo* problems (Dracup 1991). In this respect, *in vitro* seedling selection has been used by Esensee *et al.* (1991) who grew strawberry seeds of different genotypes in NaCl-amended media. Higher salt levels resulted in reduced seedling fresh and dry weights. In later report, Wright and Hughes (1993) tested different strawberry crosses for salt tolerance *in vitro* followed by *ex vitro* hydroponic testing. Seedling growth *in vitro* were more salt-tolerant than mature plants grown in hydroponics. *In vitro* plants responded best to 0.2% NaCl and some survived the 0.8% NaCl shock, while none in hydroponics did. In another studies, the number of proliferated shoots, rooting and explant survival were severely affected by the addition of NaCl to the tissue culture media (Badawi *et al.*, 1990 and Maarouf, 2001). Differences among strawberry cultivars for salt tolerance were also reported by the above investigators.

Under *in vivo* conditions, Imazu and Oosawa (1954) observed severe leaf scorch on strawberry plants grown in solution cultures containing 0.3% NaCl The growth was also depressed as the salt level was increased. Based on the incident of NaCl-induced phytotoxcity, Martinez-Barroso and Alvarez (1997) were able to distinguish between salt-tolerant and salt-sensitive strawberry genotypes. A marked reduction in the formation of new leaves and root weight was also reported on strawberry plants under salt stress (Goncharova and Dobren'Kova, 1981). In a greenhouse study, leaf numbers and inflorescence development were reduced by raising salinity, irrespective of the plant developmental stage (Awang and Atherton, 1995). In earlier report, the same workers attributed the depression of growth at high salinity to the disturbance in the uptake of essential elements and their consequent effects on photosynthesis (Awang and Atherton, 1994).

Several reports indicated that plant species and varieties differ in their response to salinity with respect to nutrients, proline or chlorophyll contents. In general, the uptake of N, K, Ca and Mg decreased by NaCl application (Hohjo *et al.*, 2001; Naidoo, 1987). It was reported by Greenway (1962) and Tal (1971) that salt tolerant cultivars contained less sodium than sensitive ones. However, results of Phills *et al.* (1979) indicated that salt tolerant tomato genotypes accumulated more Na and K and less Ca than salt sensitive genotypes. The accumulation of proline was also observed in many species as a result of exposure to salt stress. *Brassica* somaclones selected *in vitro* for salt tolerance, contained higher amount of proline than non-selected somaclones (Jain *et al.*, 1991). Regarding the effect of salinity on chlorophyll content, Sinel' nikova *et al.* (1988) showed that chlorophyll a and b and total chlorophyll decreased with salinity, and were higher in the salt tolerant tomato cultivars than in the susceptible ones. Information regarding tissue nutrients, proline or chlorophyll contents in strawberry plants in response to salinity is limited.

This study was conducted to evaluate some newly introduced strawberry cultivars for growth and other plant parameters in response to different levels of NaCl in the tissue culture medium during proliferation and rooting stages as well as during *ex vitro* growth.

MATERIALS AND METHODS

This study was conducted at the Plant Tissue Culture Laboratory and the Greenhouse Facilities of the Department of Horticulture, Faculty of Agriculture, Suez Canal University, Ismailia Governorate during the years 2000 and 2001. Six strawberry (*Fragaria* x *ananassa*) genotypes were evaluated, namely Camarosa, Carlsbad, Sweet Charlie, Seascape, Capitola and Chandler. The first five cultivars were recently introduced to Egypt for fresh plantation under plasticulture system, while Chandler is mainly grown under frigo plantation system. Strawberry cultivars response to salinity was tested *in vitro* during the proliferation and rooting stages and *ex vitro* during post-acclimatization growth period in the greenhouse.

Exp. 1. Response to Salinity During *in vitro* Proliferation.

To obtain proliferated clump for each cultivar, runner tips were collected from greenhouse-grown plants in June, 2000, sterilized with 10% (v/v) solution of clorox (5.25% NaOCl) for 5min. and rinsed three times with sterile distilled water. Meristem tips (0.5mm) were excised from the sterile runner tips and cultured directly in shoot proliferation medium containing MS (Murashige and Skoog, 1962) basal salts and vitamins were supplemented with 3% sucrose, 0.5 mg/l BA and 0.7% agar in French square jars (capacity 40ml) containing 10ml medium. Meristem-derived proliferated clumps were obtained after six weeks from culture initiation. In a trial using low salinity levels (Exp.1-a), five clumps from each cultivar were divided into single plantlets in a laminar-air flow hood and subcultured onto shoot multiplication medium as above, supplemented with NaCl treatments at 0.0, 0.05, 0.1, 0.15 and 0.2% (w/v). Three plantlets were cultured per each jar (capacity 200ml) containing 35ml of NaCl-amended medium. Cultures were incubated at 26 $\pm$ 1°C under 16hs. light (3000 lux) and 8hs. dark. The culture jars were arranged in factorial in a randomized complete blocks design with five replicates (jars). Proliferated shoot clumps under NaCl stress were removed after four weeks and data were recorded on clump fresh weight, number of plantlets per clump and number of leaves per clump. After oven-dried at 70°C for 48hs., clump dry weight was recorded. Based on these data and visual observation, none of the tested cultivars were shown to be severely affected by up to 0.2% NaCl in the medium. Therefore, a second trial was conducted (Exp. 1-b) using higher NaCl concentrations (0.3, 0.4, 0.5, 0.6, 0.7 and 0.8%). Medium composition (except NaCl levels), culture conditions, growth measurements were all the same as in Exp. 1-a. Nutrient analysis were performed using dried tissue samples from the two cultivars Carlsbad (suspected as salt-tolerant) and Chandler (as salt sensitive) under different NaCl levels in Exp.1-a. The dried proliferated clumps were ground in a stainless steel Willy Mill with a 20-mesh screen and analyzed for P, K, Ca, Mg and Na according to the methods of Peck and MacDonald (1972), and for total nitrogen by micro-kjeldahl procedure.

Exp. 2. Response to Salinity During *In Vitro* Rooting Stage.

Meristem - derived proliferated shoots were obtained as previously described. Shoots were divided into single plantlets and subcultured onto salinized rooting media containing MS basal salts and vitamins, 1.0 mg/l IBA, 3% sucrose in addition to six NaCl concentrations (0.3, 0.4, 0.5, 0.6, 0.7 and 0.8%). Media were solidified with 7g/l agar. Five plantlets were cultured per each 200ml capacity jar containing 35ml media. Culture conditions and experimental design were the same as in Exp.1. After 30 and 60 days in cultures, data were taken on a random sample of 6 rooted plantlets. Data included shoot fresh and dry weights, root length and number of roots per plantlet. Shoot tissue analysis for N, P, K, Ca, Mg and Na were performed in the two cultivars 'Carlsbad' and 'Chandler' as in Exp. 1. In addition, shoot free proline was determined by the method described by Bates *et al.* (1973).

Exp. 3. *Ex Vitro* Response to Salinity

The starting materials for this experiment were obtained by micropropagation of the six strawberry cultivars under study during the period of Oct. – Dec. 2000 and Jan.–Feb. 2001 using the technique reported by Boxus (1974). During Mar. 2001, rooted plantlets were acclimatized in the greenhouse under mist. Fully acclimatized plants were grown in 20cm plastic pots (one plant/ pot) filled with peatmoss – vermiculite mix. (1:1 v/v). Plants were watered with a solution containing one–half strength MS salts every other day. Sodium chloride treatments were added to the nutrient solution in increments of 0.0, 0.1, 0.2, 0.3, 0.4 and 0.5% (w/v) and applied to the plants on May 2, 4, 6, 8 and 10, 2001. After three weeks of the last NaCl application treatment, three replicate samples of five leaf discs were randomly taken from newly matured leaves for chlorophyll analysis according to Mackinney (1942). As shoots of most plants treated with 0.4 and 0.5% NaCl were died, growth measurements were only taken in plants under NaCl levels up to 0.3%. On July 10, 2001, plants were removed from the pots, fresh and dry weights of shoots and roots were determined on three randomly–selected plants in addition to number of runners/plant and root length. The experiment was a 6 x 5 factorial in a randomized complete blocks design. The treatments were replicated five times.

Data of all experiments were subjected to the analysis of variance procedure using SAS computer programme (SAS Institute, 1985) and mean separation using the LSD test (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

EXP.1 Response of Strawberry Cultivars to Salinity During *In Vitro* Proliferation

Exp.1-a. Response to low NaCl levels

Results of ANOVA showed significant differences among strawberry cultivars for the growth of proliferated clumps in terms of clump dry weight, number of plantlets per clump and the number of new leaves formed per clump, while the differences among cultivars were not significant for clump fresh weight (Table 1). Tested over all NaCl levels, the cultivars Carlsbad and Seascape had the highest clump dry weight, while Camarosa and Carlsbad had the highest number of plantlets and leaves/clump. A significant decrease in clump fresh weight was noticed with the increase in NaCl levels in the medium up to 0.2% for all tested cultivars but in varying degree. Compared to the control, the decrease in fresh weight at 0.2% NaCl was less than 50% for the cultivars Camarosa, Carlsbad and Seascape but more than 50% for Sweet Charlie, Capitola and Chandler. The lowest decrease in fresh weight (38%) was observed with Carlsbad and the highest (59%) was that of Chandler. This trend was almost the same for the reduction in clump dry weight with increasing NaCl levels. Significant differences among cultivars for number of plantlets per clump and number of leaves/clump were also detected (Table 1). Camarosa and Carlsbad significantly produced the highest number of plantlets, while Chandler and Sweet Charlie had the least ones. Subsequently, the number of new leaves developed per clump had the same trend.

The main effects of NaCl levels were significant for all parameters tested. Clump fresh weight, dry weight, number of plantlets/clump and number of leaves/clump at 0.2% NaCl were 49%, 41.6%, 54% and 48% less than the control, respectively. The interactions of cultivar x salt concentration were significant for clump fresh and dry weights and number of both plantlets and leaves/clump indicating that strawberry cultivars had different responses to salinity levels. For example, both Carlsbad and Seascape had higher number of plantlets at 0.15% NaCl compared to 0.1% NaCl, in contrast to the general trend of reduction in number of plantlets with each increase in NaCl levels for other cultivars.

At 0.0% NaCl, the difference among cultivars in number of plantlets/clump indicated genotypic differences in proliferation capacity. The higher the proliferation capacity of a cultivar than others at certain level of salinity would indicate the higher the degree of tolerance of that cultivar at such salinity level. The cultivars Camarosa followed by Carlsbad and Seascape exhibited higher proliferation at 0.15 and 0.2% NaCl.

Exp. 1-b. Response to high NaCl levels

In this trial, most growth parameters, especially number of plantlets and leaves per clump were inhanced at 0.3% NaCl compared to the 0.2% level used in Exp. 1-a, then started to decline at higher NaCl levels (Table 2). Although all cultivars were able to survive at NaCl levels up to 0.8%, significant differences among cultivars were observed for clump fresh and dry weight, number of plantlets and leaves/clump. Tested over all NaCl levels, Carlsbad had the highest average clump fresh weight and dry weight, while number of plantlets and/or leaves/clump were significantly the same for Carlsbad, Camarosa and Sweet Charlie (Table 2). Chandler showed severe shoot chlorosis at 0.5% and higher NaCl levels (Fig.1). Significant cultivar x salt concentration interactions were detected for clump fresh and dry weights as well as number of plantlets and leaves per clump due to different magnitude of cultivar response to NaCl levels. For example, the decline percent in clump dry weight at 0.8% NaCl relative to 0.3% was 48% for Carlsbad, while this reduction percent was 72% for Sweet Charlie. In fact, Carlsbad had the least relative decline in most growth parameters during *in vitro* multiplication stage at high salt levels, suggesting its relative tolerance to salinity among the tested cultivars. On the other hand, Chandler may be ranked as salt sensitive, based on shoot chlorosis symptoms (Fig.1) and reduction in growth (Table 2).

Tested over all cultivers, increasing NaCl level up to 0.8% resulted in a significant decrease in all growth parameters during proliferation at high NaCl levels (Table 2). The percent decline in clump fresh weight, dry weight, number of plantlets and number of leaves per clump were 64%, 56%, 63% and 43%, respectively.

The reduction in growth and proliferation and the genotypic differences observed in Exp.1 in response to NaCl stress are in agreement with the results of Badawi *et al*. (1990), Esensee *et al*. (1991), Maarouf (2001) and Wright and Hughes (1993).

Exp.2 Response of Strawberry Cultivars to Salinity During *In Vitro* Rooting Stage

Shoot and root growth of strawberry plantlets during the rooting stage were adversely affected by increasing NaCl levels but in varying degree, depending on the cultivar. From the data obtained at 30 days (Table 3), the average shoot fresh and dry weights were significantly higher for the cultivars Camarosa, Carlsbad and Seascape, and less for Sweet Charlie, Capitola and Chandler. At 0.8% NaCl, the relative decline in shoot fresh weight and dry weight was the highest for Chandler and Sweet Charlie and the least for Carlsbad.

In general, increasing NaCl level up to 0.8% in the medium significantly decreased shoot fresh weight by 69% and dry weight by 70% as compared to 0.3% NaCl (Table 3).

Significant differences among strawberry cultivars were observed for number of roots/plantlet and the average root length. Seascape had the highest number of roots followed by Camarosa and Carlsbad, while differences in root number per plantlets were not significant among the less rooting cultivars (Sweet Charlie, Capitola and Chandler). At the highest NaCl level (0.8%), Seascape followed by Carlsbad had the largest root number and length, while Chandler had the least ones. These results indicated the relative tolerance of the cultivars Seascape and Carlsbad to high salinity levels in terms of *in vitro* rooting capacity.

Regarding the main effects of NaCl on rooting, a marked reduction in root number and root length/plantlet was detected with increasing NaCl levels (Table 3 and Fig. 2). At 0.8% NaCl, this reduction was 80% for root number and 88% for root length as compared to rooting at 0.3% NaCl. The interactive effects of cultivar x salt concentration were significant for all growth parameters at 30 days.

Growth parameters of strawberry cvs which responded to increase NaCl level in the rooting medium were also measured at the termination of the rooting experiment after 60 days (Table 4). Results, generally, showed that longer exposure to salt stress *in vitro* did not change the trends previously noted at 30 days, neither for the main effects of salts nor the interactions between cultivars and NaCl levels. However, most growth parameters were increased at 60 days compared to those measured at 30 days. This may be due to reaching maximum utilization of nutrients from the media with time.

The results of this experiment agreed with those of Esensee *et al.* (1991) and Wright and Hughes (1993) who tested different strawberry genotypes for salt tolerance *in vitro* at the seedling stage.

Based on the growth data obtained during the *in vitro* proliferation and rooting experiments under salt stress, the strawberry cultivar 'Carlsbad' could be ranked as the most likely cultivar to be salt tolerant, while 'Chandler' being the most sensitive to salt among the six tested cultivars. The differences between these two cultivars in nutrients uptake and proline accumulation have been tested further.

Nutrient contents in response to salinity during *in vitro* proliferation

Tissue nutrient contents of the cultivars Carlsbad and Chandler during proliferation at 0.0 – 0.2% NaCl are presented in Fig.3. Results indicated significant differences between the two cultivars for tissue N, P, K, Ca and Na contents, while the differences for Mg content were not significant. The cultivar Carlsbad had significantly more N, K and Ca but less P and Na than Chandler. Results also showed that with the increase in NaCl levels in the medium, significant reduction in tissue N, P and K were detected, while the reverse was true for tissue Ca, Mg, and Na contents. The cultivar x salt concentration interactions were significant for all nutrients tested except for tissue Mg as shown in Fig.3. These significant interactions demonstrated that levels of salinity had different influence and the cultivars had different response. For Carlsbad, the percent decline in N, P and K at 0.2% NaCl relative to the control were 31%. 26% and 13%, respectively. However, for Chandler, these values were 62%, 42% and 38%, respectively.

Nutrient contents in response to salinity during *in vitro* rooting

Results in Fig.4 showed significant differences between the two cultivars Carlsbad and Chandler for tissue N, P, K, Ca and Na contents when tested over all salt levels. Carlsbad had more contents of N, K and Ca but less P and Na than Chandler, while Mg content was the same for both cultivars during the *in vitro* rooting. Tested over the two cultivars, tissue N, K and Ca decreased, while Mg and Na increased with the increment in NaCl levels. The leaf tissue P increased with elevated levels of NaCl up to 0.6%, then declined (Fig.4). The interactions of cultivars x salt were significant for all nutrients which was clearly demonstrated in Fig.4.

Results of nutrient contents in salt tolerant and salt sensitive cultivars during rooting, generally, confirmed the results obtained during proliferation stage. It was clear that salt tolerant cultivar had more efficient nutrient utilization than salt sensitive one under salinity stress. This was in accordance with the results of Awang and Atherton (1994) which would explain the better growth performance of Carlsbad cv and the depression of growth of Chandler cv under salt stress. The enhanced growth of Carlsbad may also be attributed to its ability to restrict Na and Mg translocation to the leaves. The higher accumulation of Na in salt sensitive cultivar was previously noted by Greenway (1962) and Tal (1971). Sodium Chlorid – induced reduction in N, K and Ca was also reported by Naidoo (1987) and Hohjo *et al.* (2001). The reduction in tissue N under saline condition was attributed to the NH^+_4/Na^+ competition (Naidoo, 1987).

Proline content in response to salinity during *in vitro* rooting

Significant differences between the two cultivars Carlsbad and Chandler were detected in proline content (Fig.5). Carlsbad had significantly higher proline than Chandler. In addition, proline increased sharply in the tissues with the increase in NaCl levels in the medium. Proline was almost nine times higher at 0.7% compared to the control. The interaction of cultivar x salt was significant. As shown in Fig.5, proline content was higher in the tissues of Chandler cv at 0.0, 0.3 and 0.4% NaCl than Carlsbad. However, at higher NaCl levels, tissues of Carlsbad accumulated more proline. It has been suggested that proline acts as endogenous osmoregulant and protects enzymes against conformation caused by mineral ions (Lerner, 1985). The finding that proline accumulated more in salt tolerant cultivar was in agreement with the results of Jain *et al.* (1991) and may support our view that Carlsbad is relatively salt tolerant cultivar as compared to Chandler.

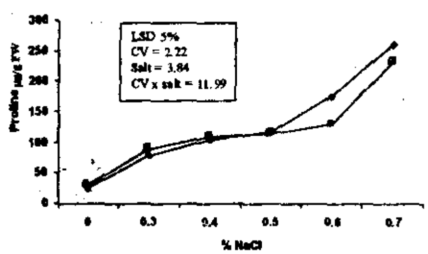

Fig.5: Tissue proline content of the strawberry cultivars Carlsbad (•) and chandler (♦) in response to different NaCl levels during *in vitro* rooting stage.

Exp.3 *Ex Vitro* Response of Strawberry Cultivars to Salinity:

Results of the responses of six strawberry cultivars to salinity during greenhouse growth are shown in Tables 5 and 6. Considering the main effect of cultivars, Capitola cv had the least shoot fresh and dry weight, compared to the other cultivars. In addition, Chandler had the highest average root fresh and dry weight and Camarosa had the highest root length when tested over all salt levels. (Table 5). All growth parameters significantly decreased with the increase in NaCl levels (Table 5). Significant cultivar x salinity interactions were detected for all growth parameters *ex vitro* (Table 6). At 0.3% NaCl, Seascape followed by Carlsbad had the highest shoot fresh weight, Camarosa and Carlsbad had the highest shoot dry weight. Results also showed that seascape had the highest root fresh weight and Carlsbad had the highest root dry weight, while Camarosa and Seascape had greater root length than the other cultivars at 0.3% NaCl. However, differences among cultivars for runner number were not detected at 0.3% NaCl (Table 6). After four weeks of growth in the greenhouse, none of the tested strawberry cultivars survived the NaCl application levels higher than 0.3%. This trend was different than the results obtained under *in vitro* conditions (Exp.1, 2) where most cultivars survived in different degree under NaCl treatments up to 0.8%. The same finding was reported by Wright and Hughes (1993). The observed differences among strawberry genotypes in response to salinity treatment *in vivo*, and the reduction in growth parameters with elevated levels of NaCl were in accordance with the results of Awang and Atherton (1995), Goncharova and Dobren'Kova (1981), Imazu and Oosawa (1954), Martinez-Barroso and Alvarez (1997). The better performance of the cultivars Camarosa, Carlsbad and Seascape under high salinity levels in the greenhouse was similar to the results obtained under *in vitro* conditions.

Chlorophyll content *ex vitro*

Significant differences among the tested strawberry cultivars were detected for chlorophyll content (Table 7). The cultivar Seascape had significantly higher chlorophyll a, b and total chlorophyll than the other cultivars. At the highest level of

NaCl, Seascape followed by Carlsbad had the highest chlorophyll a, b and total. Considering the main effect of salt level, both chlorophyll a and b as well as total chlorophyll decreased significantly with each increase in NaCl level. Significant cultvar x salt interactions were detected for chlorophyll contents (Table 7). At 0.5% NaCl, Carlsbad had 37% less chlorophyll than the control, while for Chandler this value was 80.7%, indicating that the salt–tolerant cultivar will maintain higher tissue chlorophyll contents than the susceptible one under salinity stress. Similar results were reported by Sinel'nikova *et al.* (1988).

In conclusion, the obtained results indicated that the *in vitro* procedure may be an effective and rapid way to screen large number of strawberry mericlones for salt tolerance followed by greenhouse situations that substantiated the *in vitro* studies. However, the *in vitro* reactions to salinity might not be the same as for plant grown *in vivo*. Under two stages of *in vitro* growth in NaCl-amended media, the newly introduced strawberry cultivar 'Carlsbad' generally showed better growth, higher N, K and less Na uptake, and higher proline accumulation which confirmed its relative tolerance to salinity as compared to 'Chandler'. Seascape may also be ranked as salt-tolerant cultivar based on its *in vitro* and *ex vitro* growth performance and its high chlorophyll content under salinity stress.

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Table 1: *In vitro* growth response of six strawberry cultivars grown under different NaCl levels during proliferation stage (Exp.1-a)

Cultivar	NaCl in the medium (%)					Mean
	0.0	0.05	0.1	0.15	0.2	
			a- Clump fresh weight (g)			
Camarosa	2.533	1.700	1.770	1.900	1.350	1.850
Carlsbad	2.226	1.936	1.763	2.100	1.383	1.882
Sweet Charlie	2.186	1.776	1.026	1.420	0.960	1.473
Seascape	2.353	1.796	1.483	1.193	1.190	1.603
Capitola	2.136	1.830	1.810	1.630	1.110	1.703
Chandler	2.240	1.920	1.556	1.476	0.916	1.677
Mean	2.312	1.826	1.568	1.569	1.151	
LSD 5% :	CV = NS,		Salt = 0.340,		CV x salt = 0.481	
			b- Clump dry weight (g)			
Camarosa	0.145	0.133	0.125	0.122	0.116	0.128
Carlsbad	0.194	0.165	0.159	0.143	0.122	0.156
Sweet Charlie	0.228	0.146	0.143	0.114	0.110	0.148
Seascape	0.235	0.175	0.169	0.162	0.149	0.178
Capitola	0.206	0.138	0.136	0.135	0.105	0.144
Chandler	0.174	0.161	0.155	0.139	0.092	0.144
Mean	0.197	0.153	0.148	0.136	0.115	
LSD 5% :	CV = 0.004,		Salt = 0.003,		CV x salt = 0.005	
			c- No. of plantlet / clump			
Camarosa	19.66	12.66	11.00	10.00	10.00	12.664
Carlsbad	16.33	9.66	8.33	11.66	7.33	10.662
Sweet Charlie	11.33	10.33	8.66	7.66	5.66	8.728
Seascape	14.33	12.66	5.33	8.66	6.33	9.462
Capitola	14.00	12.00	8.66	4.66	5.33	8.930
Chandler	12.00	8.33	6.66	5.66	5.66	7.662
Mean	14.608	10.94	8.106	8.05	6.718	
LSD 5% :	CV = 1.560,		Salt = 1.430,		CV x salt = 2.026	
			d- No. of leaves / clump			
Camarosa	34.00	20.00	19.30	18.00	15.33	21.467
Carlsbad	28.66	16.00	16.33	17.00	12.33	18.06
Sweet Charlie	21.33	23.33	12.00	15.33	12.66	16.93
Seascape	24.00	22.00	12.66	18.00	12.00	17.73
Capitola	20.33	26.66	13.00	10.00	9.66	15.93
Chandler	19.33	12.33	14.66	12.66	14.33	14.66
Mean	24.60	20.05	14.66	15.16	12.71	
LSD 5% :	CV = 3.058,		Salt = 2.79,		CV x salt = 4.488	

Table 2: *In vitro* growth response of six strawberry cultivars grown under different NaCl levels during proliferation stage (Exp.1-b)

Cultivar	NaCl in the medium (%)						Mean
	0.3	0.4	0.5	0.6	0.7	0.8	
			a- Clump fresh weight (g)				
Camarosa	1.820	1.666	1.400	1.083	0.806	0.633	1.235
Carlsbad	2.030	1.820	1.186	1.063	1.063	1.016	1.363
Sweet Charlie	1.986	1.283	1.220	0.976	1.880	0.406	1.126
Seascape	1.660	0.776	0.643	0.600	0.426	0.510	0.771
Capitola	1.463	1.270	1.040	0.936	0.763	0.633	1.018
Chandler	1.363	1.166	1.303	0.813	0.600	0.533	0.963
Mean	1.721	1.320	1.143	0.913	0.757	0.622	
LSD 5% :	CV = 0.212,		Salt = 0.212,		CV x salt = 0.301		
			b- Clump dry weight (g)				
Camarosa	0.155	0.135	0.110	0.093	0.085	0.070	0.1080
Carlsbad	0.169	0.140	0.129	0.120	0.091	0.088	0.1226
Sweet Charlie	0.172	0.123	0.107	0.096	0.078	0.048	0.1040
Seascape	0.122	0.090	0.070	0.073	0.071	0.052	0.0796
Capitola	0.141	0.115	0.102	0.086	0.085	0.069	0.0996
Chandler	0.138	0.112	0.112	0.089	0.085	0.066	0.1003
Mean	0.1496	0.1193	0.1051	0.0930	0.0826	0.0655	
LSD 5% :	CV = 0.0009		Salt = 0.0009		CV x salt = 0.0013		
			c- No. of plantlet / clump				
Camarosa	12.00	8.00	7.00	6.00	5.33	4.33	7.110
Carlsbad	12.00	9.66	7.66	5.33	5.66	5.66	7.660
Sweet Charlie	11.33	7.66	7.66	6.33	6.00	4.00	7.163
Seascape	10.33	6.33	4.66	5.33	4.33	3.00	5.663
Capitola	8.00	6.66	6.00	5.66	3.66	3.00	5.496
Chandler	8.00	7.00	6.00	5.66	3.66	2.66	5.496
Mean	10.276	7.551	6.496	5.718	4.773	3.775	
LSD 5% :	CV = 2.32,		Salt = 2.324,		CV x salt = 1.720		
			d- No. of leaves / clump				
Camarosa	20.66	18.00	16.66	15.66	12.00	10.33	15.556
Carlsbad	24.66	21.33	14.33	12.66	12.00	10.66	15.944
Sweet Charlie	21.33	20.00	17.00	13.00	13.33	10.66	15.889
Seascape	23.66	15.66	12.66	13.33	12.33	11.66	14.889
Capitola	20.33	15.33	15.32	12.00	10.33	9.66	13.833
Chandler	15.00	12.66	13.00	13.30	9.00	7.66	11.778
Mean	20.944	17.167	14.833	13.333	11.498	10.11	
LSD 5% :	CV = 1.787,		Salt = 1.787,		CV x salt = 2.535		

Table 3: *In vitro* growth response of six strawberry cultivars grown under different NaCl levels during the rooting stage at 30 days.

Cultivar	NaCl in the medium (%) 0.3	0.4	0.5	0.6	0.7	0.8	Mean
			a- Plantlet fresh weight (g)				
Camarosa	1.103	1.196	1.103	0.913	0.646	0.396	0.893
Carlsbad	1.123	1.933	0.730	0.513	0.463	0.513	0.713
Sweet Charlie	1.016	0.930	0.666	0.416	0.330	0.223	0.597
Seascape	1.190	1.080	0.573	0.470	0.536	0.416	0.710
Capitola	0.740	0.610	0.653	0.393	0.403	0.243	0.507
Chandler	1.123	0.690	0.750	0.536	0.246	0.166	0.585
Mean	1.049	0.925	0.746	0.540	0.437	0.326	
LSD 5% :	CV = 0.163		Salt = 0.163,		CV x salt = 0.232		
			b- Plantlet dry weight (g)				
Camarosa	0.140	0.133	0.126	0.090	0.063	0.050	0.1005
Carlsbad	0.126	0.120	0.100	0.070	0.063	0.053	0.0880
Sweet Charlie	0.110	0.080	0.070	0.050	0.033	0.026	0.0610
Seascape	0.146	0.103	0.060	0.053	0.050	0.036	0.0746
Capitola	0.083	0.073	0.076	0.053	0.056	0.026	0.0485
Chandler	0.110	0.080	0.083	0.060	0.033	0.026	0.0653
Mean	0.102	0.098	0.086	0.062	0.049	0.036	
LSD 5% :	CV = 0.004		Salt = 0.004		CV x salt = 0.0058		
			c- No. of roots / plantlet				
Camarosa	18.66	13.66	13.00	10.66	5.33	3.00	10.722
Carlsbad	14.00	11.33	10.33	7.33	6.33	4.00	8.889
Sweet Charlie	18.00	17.00	14.30	7.66	4.00	2.30	10.556
Seascape	20.66	18.33	12.33	9.33	8.66	6.33	12.610
Capitola	12.00	12.30	13.30	8.66	4.00	2.66	8.830
Chandler	16.66	10.00	13.00	8.33	3.00	1.00	8.880
Mean	16.66	13.77	12.72	8.66	5.22	3.22	
LSD 5% :	CV = 2.32,		Salt = 2.324,		CV x salt = 3.296		
			d- Root length (cm)				
Camarosa	10.40	9.66	7.16	7.66	4.70	1.30	6.813
Carlsbad	13.83	12.00	10.50	6.66	3.70	1.86	8.091
Sweet Charlie	10.33	9.10	8.66	2.40	1.26	0.83	5.430
Seascape	11.00	10.70	9.66	8.33	5.66	2.86	8.039
Capitola	9.76	8.83	8.00	5.00	3.13	0.86	6.017
Chandler	11.93	9.10	8.00	6.93	3.80	0.36	6.686
Mean	11.21	9.89	8.66	6.16	3.71	1.34	
LSD 5% :	CV = 1.11		Salt = 1.112		CV x salt = 1.577		

Table 4: *In vitro* growth response of six strawberry cultivars grown under different NaCl levels during the rooting stage at 60 days.

Cultivar	NaCl in the medium (%)						Mean
	0.3	0.4	0.5	0.6	0.7	0.8	
			a- Plantlet fresh weight (g)				
Camarosa	1.626	1.370	0.813	0.793	0.650	0.383	0.939
Carlsbad	1.146	0.973	0.643	0.783	0.510	0.346	0.734
Sweet Charlie	0.996	1.000	0.980	0.810	0.243	0.243	0.714
Seascape	1.233	1.536	0.920	0.653	0.290	0.316	0.825
Capitola	0.966	1.073	0.790	0.830	0.670	0.290	0.770
Chandler	1.450	1.126	0.967	0.640	0.163	0.126	0.746
Mean	1.237	1.181	0.851	0.752	0.421	0.284	
LSD 5% :	CV = 0.197,		Salt = 0.197,			CV x salt = 0.280	
			b- Plantlet dry weight (g)				
Camarosa	0.166	0.155	0.080	0.080	0.066	0.060	0.1013
Carlsbad	0.116	0.110	0.086	0.065	0.060	0.035	0.0790
Sweet Charlie	0.093	0.090	0.066	0.065	0.036	0.033	0.0638
Seascape	0.124	0.121	0.103	0.063	0.043	0.031	0.0810
Capitola	0.096	0.110	0.090	0.088	0.060	0.046	0.0816
Chandler	0.140	0.120	0.083	0.063	0.023	0.009	0.0730
Mean	0.1226	0.1178	0.084	0.0708	0.048	0.035	
LSD 5% :	CV = 0.0024,		Salt = 0.0024,			CV x salt = 0.0034	
			c- No. of roots / plantlet				
Camarosa	22.33	20.66	15.00	15.00	10.66	8.66	15.385
Carlsbad	21.33	18.00	15.66	13.66	10.00	6.66	14.218
Sweet Charlie	16.00	15.30	14.00	11.00	8.30	7.00	11.933
Seascape	23.66	20.33	16.33	12.66	8.33	7.66	14.828
Capitola	17.00	16.00	14.00	13.66	8.00	4.00	12.110
Chandler	18.60	17.00	15.00	14.30	5.66	2.60	12.193
Mean	19.82	17.88	14.99	13.38	8.49	6.09	
LSD 5% :	CV = 3.12,		Salt = 3.12,			CV x salt = 4.428	
			d- Root length (cm)				
Camarosa	19.50	14.83	14.66	14.30	5.83	4.33	12.24
Carlsbad	22.16	13.46	18.16	14.16	12.66	9.66	15.04
Sweet Charlie	19.50	15.66	10.20	5.16	5.33	4.00	9.89
Seascape	19.00	21.00	16.66	12.30	9.83	7.00	14.30
Capitola	16.20	16.16	13.56	12.60	5.66	1.66	10.97
Chandler	11.66	11.66	12.00	10.16	2.86	1.20	8.25
Mean	18.00	15.46	14.20	11.46	7.02	4.64	
LSD 5% :	CV = 2.42,		Salt = 2.42,			CV x salt = 3.434	

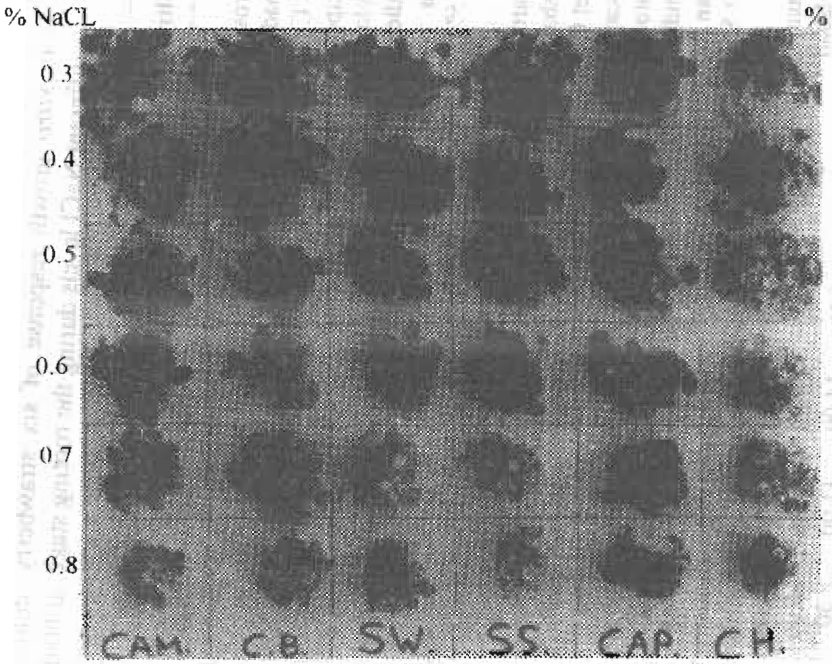

Fig. 1

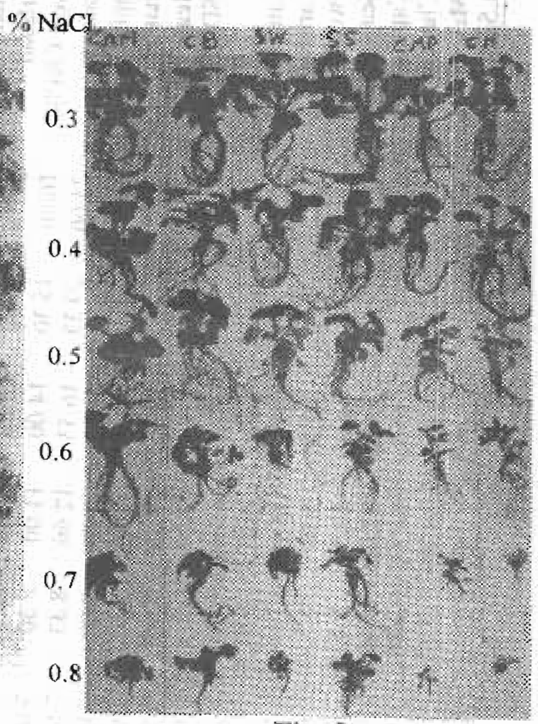

Fig. 2

Fig. 1. Cultivar response to NaCl during *in vitro* proliferation. *
Fig. 2. Cultivar response to NaCl during *in vitro* rooting.*
* From left to right: Camarosa, Carlsbad, Sweet Charlie, Seascape, Capitola, and Chandler.

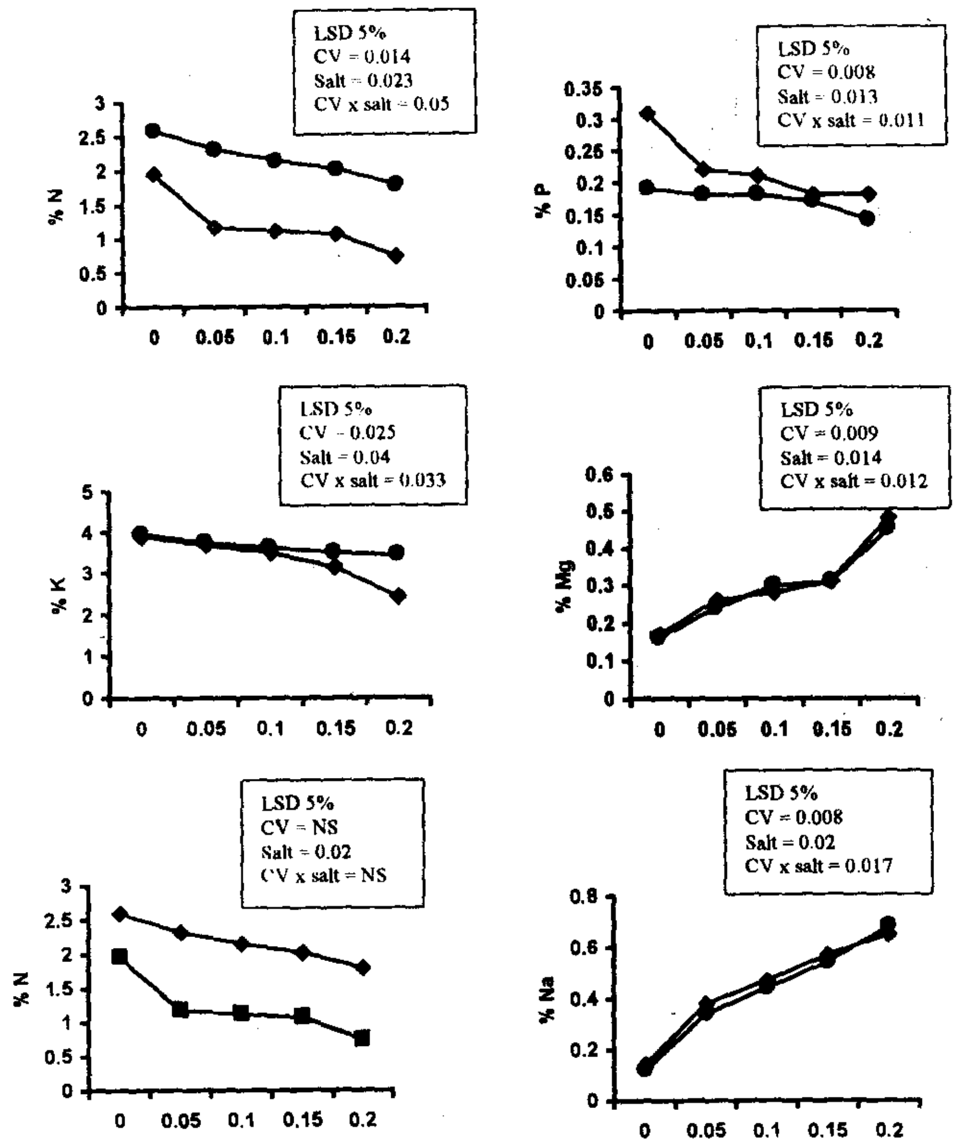

Fig. 3: Tissue nutrient contents of the strawberry cultivars Carlsbad (•) and Chandler (♦) in response to NaCl levels in the media during the proliferation stage.

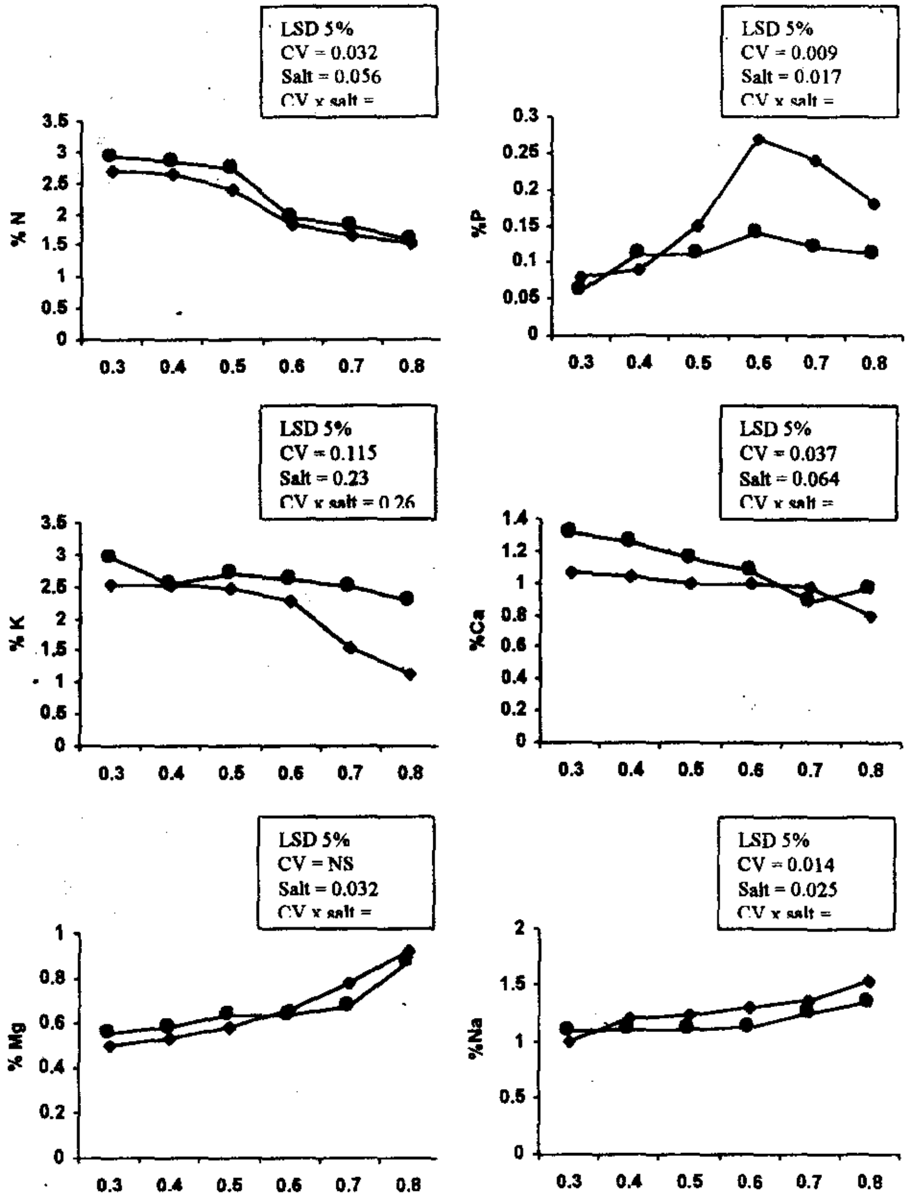

Fig. 4: Tissue nutrient contents of the strawberry cultivars Carlsbad (•) and Chandler (♦) in response to NaCl levels in the media during the rooting stage.

Table 5: Main effects of strawberry cultivars and NaCl levels on the greenhouse growth characters.

Treatment		Growth Character*					
		SFW[1] (g)	SDW[2] (g)	RFW[3] (g)	RDW[4] (g)	RL[5] (cm)	Runner (no.)
a- Main effect of cultivars							
Camarosa		5.100	1.185	0.950	0.2028	11.750	1.853
Carlsbad		5.933	1.105	1.250	0.1661	9.167	1.500
Sweet Charlie		6.025	1.224	1.242	0.2047	9.083	1.833
Seascape		6.242	1.267	1.450	0.1943	10.083	1.333
Capitola		3.775	0.838	0.517	0.1369	11.167	2.083
Chandler		6.225	1.142	1.758	0.2542	8.500	1.667
LSD 5% :		1.061	0.347	0.306	0.0346	0.889	0.443
b- Main effect of NaCl levels							
% NaCl	0.0	7.538	1.590	1.556	0.254	11.722	2.50
	0.1	6.227	1.343	1.294	0.219	10.278	1.83
	0.2	4.994	0.980	1.078	0.174	9.556	1.44
	0.3	3.439	0.594	0.850	0.125	8.278	1.05
LSD 5%		0.867	0.284	0.250	0.028	0.726	0.362

* 1 = shoot fresh weight, 2 = shoot dry weight, 3 = root fresh weight, 4 = root dry weight, 5 = root length.

Table 6: Interaction effects of strawberry cultivars and NaCl levels on the greenhouse growth characters.

Treatment		Growth Character*					
Cultivar	% NaCl	SFW[1] (g)	SDW[2] (g)	RFW[3] (g)	RDW[4] (g)	RL[5] (cm)	Runner (no.)
Camarosa	0.0	6.30	1.493	1.30	0.237	13.66	2.66
	0.1	5.83	1.288	0.96	0.228	12.00	2.00
	0.2	4.93	1.032	0.86	0.201	12.00	1.66
	0.3	3.33	0.925	0.66	0.144	9.33	1.00
Carls bad	0.0	7.13	1.364	1.53	0.178	9.66	2.30
	0.1	6.80	1.287	1.30	0.173	9.33	1.66
	0.2	5.20	0.890	1.10	0.150	9.00	1.00
	0.3	4.60	0.879	1.06	0.162	8.66	1.00
Sweet Charlie	0.0	8.56	1.796	1.50	0.271	11.66	3.30
	0.1	6.93	1.314	1.40	0.237	9.33	1.66
	0.2	5.26	1.250	1.13	0.184	8.00	1.33
	0.3	3.33	0.538	0.93	0.126	7.33	1.00
Seascape	0.0	8.53	1.970	1.63	0.280	11.33	1.66
	0.1	5.83	1.852	1.56	0.229	10.00	1.33
	0.2	5.40	0.829	1.33	0.166	9.66	1.33
	0.3	5.20	0.417	1.26	0.101	9.33	1.00
Capitola	0.0	4.80	1.159	0.73	0.168	13.66	2.66
	0.1	4.13	0.938	0.56	0.143	11.66	2.33
	0.2	3.83	0.748	0.43	0.126	10.66	2.00
	0.3	2.33	0.507	0.33	0.109	8.66	1.33
Chandler	0.0	9.90	1.763	2.63	0.392	10.33	2.33
	0.1	7.83	1.375	1.96	0.302	9.33	2.00
	0.2	5.33	1.130	1.60	0.215	8.00	1.33
	0.3	1.83	0.298	0.83	0.107	6.33	1.00
LSD 5%		1.232	0.512	0.412	0.041	1.46	0.662

* 1 = shoot fresh weight, 2 = shoot dry weight, 3 = root fresh weight, 4 = root dry weight, 5 = root length.

Table 7: Leaf chlorophyll contents of six strawberry cultivars grown under different NaCl levels in the greenhouse.

Cultivar	NaCl %						Mean
	0.0	0.1	0.2	0.3	0.4	0.5	
			a- Chlorophyll a (mg/g FW)				
Camarosa	1.329	1.094	0.976	0.869	0.680	0.579	0.922
Carlsbad	1.811	1.568	1.460	1.219	1.095	0.955	1.352
Sweet Charlie	3.161	1.834	1.432	1.091	0.862	0.751	1.522
Seascape	2.252	2.138	2.052	1.888	1.645	1.744	1.953
Capitola	2.635	1.949	1.432	0.982	0.603	0.573	1.363
Chandler	2.307	1.736	1.358	0.948	0.711	0.471	1.255
Mean	2.250	1.720	1.452	1.167	0.933	0.846	
LSD 5% :	CV = 0.435,		Salt = 0.435,		CV x salt = 0.672		
			b- Chlorophyll b (mg/g FW)				
Camarosa	0.570	0.445	0.575	0.680	0.460	0.385	0.520
Carlsbad	0.761	0.612	0.747	0.538	0.559	0.611	0.639
Sweet Charlie	1.511	0.731	0.718	0.462	0.408	0.428	0.710
Seascape	0.997	1.111	0.919	0.893	0.693	0.735	0.892
Capitola	1.720	0.858	0.567	0.538	0.324	0.468	0.746
Chandler	1.746	1.129	0.640	0.553	0.542	0.311	0.820
Mean	1.218	0.815	0.695	0.611	0.498	0.490	
LSD 5% :	CV = 0.232,		Salt = 0.232,		CV x salt = 0,362		
			c- Total Chlorophyll (mg/g FW)				
Camarosa	1.899	1.540	1.219	1.550	0.885	0.965	1.343
Carlsbad	2.572	2.185	2.200	1.757	1.655	1.331	1.952
Sweet Charlie	4.670	2.565	2.151	1.554	1.268	1.179	2.232
Seascape	3.250	3.249	2.971	2.771	2.338	2.479	2.843
Capitola	4.355	2.807	2.000	1.521	0.928	1.039	2.109
Chandler	4.053	2.599	1.998	1.501	1.253	0.782	2.031
Mean	3.467	2.491	2.091	1.776	1.388	1.296	
LSD 5% :	CV = 0.627,		Salt = 0.627,		CV x salt = 0.842		

استجابة ستة أصناف من الفراولة للملوحة خلال مرحلتى التبرعم الخضرى والتجذير بمزارع الأنسجة وخلال مرحلة ما بعد الأقلمة بالصوبة

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تم اختبار ستة أصناف من الفراولة وهى كماروزا وكارلسباد وسويت شارلى وسيسكاب وكابيتولا وشندلر تحت مستويات مختلفة من الملوحة (صفر - ٠,٨% كلوريد صوديوم) فى بيئة مزارع الأنسجة خلال مرحلتى التبرعم والتجزير، وأتبعها اختبار لمدى التحمل للملوحة تحت ظروف الصوبة لنفس الأصناف. أظهرت النتائج فى تجارب مزارع الأنسجة وجود اختلافات معنوية بين الأصناف لمدى تحملها للملوحة، واعتمادا على صفات النمو المختلفة مثل الوزن الطازج والجاف للأجزاء الخضرية، والقدرة على التبرعم الخضرى، والتجزير تحت ظروف الملوحة فى البيئة، فقد أظهر الصنف كارلسباد أعلى قدرة على تحمل الملوحة بينما كان الصنف شندلر أكثر الأصناف المختبرة حساسية للملوحة تحت ظروف المعمل. وعند تحليل محتوى الأنسجة من العناصر المعدنية والبرولين فى الصنفين تحت تركيزات مختلفة من الملوحة، فقد وجد أن أنسجة الصنف كارلسباد تحتوى على تركيزات أعلى من النتروجين والبوتاسيوم والكالسيوم والبرولين وتركيز أقل من الصوديوم، مقارنة بالصنف شندلر مما يؤكد القدرة النسبية للصنف كارلسباد على تحمل الملوحة. كما أوضحت النتائج أن زيادة تركيز كلوريد الصوديوم فى البيئة نتج عنه نقص معنوى فى كل صفات النمو خلال مرحلتى التبرعم والتجزير، ونقص فى محتوى الأنسجة من عناصر النتروجين والبوتاسيوم والكالسيوم مع زيادة فى تركيز الصوديوم والمغنسيوم والبرولين. وبالنسبة لاستجابة الأصناف للملوحة تحت ظروف الصوبة، فقد كانت النتائج متشابهة تقريبا مع تلك تحت ظروف مزارع الأنسجة. وكان محتوى الأوراق من الكلوروفيل أعلى فى الصنفين كارلسباد وسيسكاب عن باقى الأصناف تحت المستويات الأعلى من كلوريد الصوديوم. ولوحظ نقص معنوى فى تركيزات الكلوروفيل مع كل زيادة فى معدل إضافة كلوريد الصوديوم. هذا ولم تستمر حيوية النباتات المعاملة بتركيزات كلوريد الصوديوم الأعلى من ٠,٣% تحت ظروف الصوبة، على عكس تلك التى تحملت المعاملة بتركيزات حتى ٠,٨% بمزارع الأنسجة مما يوضح اختلاف النباتات فى الاستجابة للملوحة حسب أطوار نموها.