EFFECTS OF CALCIUM FORMS ON ELECTROLYTE LEAKAGE, TOTAL NITROGEN, YIELD AND BIOMASS PRODUCTION BY STRAWBERRY PLANTS UNDER NACL SALINITY

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ABSTRACT

Strawberry (Fragaria ananassa Duch.) plants cv. Selva was grown in soilless culture under greenhouse conditions to present the effects of application of supplementary calcium combined with nutrient solution on plants grown under NaCl (35 mmol) salinity. Treatments: (1) Commercial nutrient solution or control; (2) the addition of NaCl (35 mmol); (3) NaCl (35 mmol) + $CaCl_2$ (5 mmol); (4) NaCl (35 mmol) + $CaCl_2$ (10 mmol); (5) NaCl (35 mmol) + $CaSO_4$ (5 mmol); (6) NaCl (35 mmol) +$CaSO_4$ (10 mmol). Data showed that NaCl stress strongly decreased leaf and runner number, flower production, fruit set, shoot and root fresh weight, total yield and calcium contents of root. On the other hand, it was found that calcium salts had an impact on negative effects of NaCl stress on these variables and reduced it. These results showed that calcium sulfate was better than calcium chloride ameliorating the negative effects of NaCl salinity on this cultivar.

Key words: biomass, electrolyte leakage, NaCl stress, supplementary calcium, total nitrogen.

INTRODUCTION

Saline water occupies 71% of the Earth area. It is thought that even a quarter of the whole pedospher is affected by salts [11], amounting to 950 × 10^6 ha [10], while 23% of the 1.5 × 10^9 ha cultivated land is considered as saline [23]. The increasing population and urbanization have forced agricultural producers to utilize marginal lands that are often saline. Thus they must use lower quality of water while minimizing the potential contamination of water resources [2]. One of the basic environmental factors mainly in arid and semi-arid regions is salinity stress that depresses crop productivity [19]. Sonneveled et al. [26] found that the total concentration of salts in nutrient solution has a strong impact on commercial yield of crops under hydroponic systems. Salinity stress alters physiological activities of plant via effect on nutrient availability, competitive uptake, transportation or partitioning within the plant organs [12] or by water deficiency and ionic imbalance [6 & 17]. It has cleared that Na^+ ion compete with Ca^{2+} ion for binding sites under salinity conditions, and it has shown that calcium ameliorates the negative effects of sodium stress on plant growth and development [9 & 17]. The main aims of this study were to investigate on the effects of salinity on strawberry growth and development and comparing two forms of calcium salts to reduce the negative effects of NaCl under salinity stress.

MATERIAL AND METHODS

Planting conditions

This pot experiment was conducted on strawberry plants cv. Selva in soilless culture under greenhouse conditions. Cold stored and bare-rooted plants each had a well-developed crown with 8-10 mm diameter were planted into perlite contained pots on April 2007. Day/night temperature ranged between 20-25/15-17 ºC. Plants were supplied by a commercial solution (Melspray containing 20-20-20 combination of N-P-K) four times a day, and pH of solution was adjusted in 5.5-6.5 using nitric acid, and its concentration was 800-1000 mgL^{-1}. The establishment period lasted from April to May, and treatments were done then after.

Treatments included:

1) Commercial nutrient solution or control [expressed as (N)];

2) Addition of NaCl (35 mmol) [expressed as (NS)];

3) NaCl (35 mmol) + $CaCl_2$ (5 mmol) [expressed as (NS1)];

4) NaCl (35 mmol) + $CaCl_2$ (10 mmol) [expressed as (NS2)];

5) NaCl (35 mmol) + $CaSO_4$ (5 mmol) [expressed as (NS3)];

6) NaCl (35 mmol) + $CaSO_4$ (10 mmol) [expressed as (NS4)].

These treatments were done on established plants one month after cultivation date (on May) and lasted to October 15, 2007.

Leaf area (LA) and runner number (RN): These variables were assessed on 6 plants per treatment. Expanded leaves each with 3 leaflets were used to account leaf area.

Electrolyte leakage (EL): This variable was used to estimate the membrane permeability, using an electrical conductivity meter. Assessments were done using six plants per treatment. Four samples were taken and cut into 1-cm^2 segments. The prepared samples were placed in individual blocked vials containing 10ml of distilled water, after three washes with distilled water removing surface contamination. Consequently, these samples were incubated at room temperature (25 ºC) on a shaker with 100 rpm for 24h, and electrolyte leakage of bathing solutions was read at once and showed as EC1. The same samples were placed in an autoclave at 120 ºC for 20 min and the second reading was taken after which the solution cooled to room temperature. This reading showed as EC2. Finally, the electrolyte leakage calculated as EC1/ EC2 ratio and expressed as percent [20].

Relative water content (RWC): Four leaf disks each in 1.5 cm^2 areas were prepared in each replication of each treatment. The fresh weight of each disk measured immediately (W), and then sample was hydrated with deionized water to full turgidity under normal room temperature and light for 4h. After this time, sample took out of water and was well dried of any surface moisture quick and lightly using filter paper. The weight of yielded sample was measured to obtain fully turgid weight (TW). Samples were oven-dried at 80 ºC for 24h separately and weighted to determine dry weight (DW). These data were used in following formula and answers showed as percent [4].

$$RWC\ (\%) = [(W-DW)/ (TW-DW)]\times 100$$

W= sample fresh weight (g); TW= sample turgid weight (g); DW= sample dry weight (g).

Proline amount (PA), total nitrogen (TN) and crude protein rate (CPR): Free proline was calculated using method of Bates et al. [5]. Total nitrogen was assessed with microkjeldahl method. Crude protein determined by method of A.O.A.C. [1], and data used in following formula:

$$\text{Crude protein } (\%) = \text{Total nitrogen} \times 6.25$$

Special leaf area (SLA) and special leaf weight (SLW): Ten disks in 1.76 cm^2 areas were prepared from each

replication in each treatment. Separated disks placed in 100 ºC oven for 48h to lose water and dried samples weighted instantly [14]. The following formulas were used:

SLW = Disk dry weight/1.76

SLA = 1.76/disk dry weight

Biomass production (BP): Shoot and root fresh weights were measured at the end of experimental period. The same plant parts were oven-dried at 80 ºC for 48h and used to measure dry weight.

Nutrient analysis (NA): Oven-dried materials of both shoot and root of each replication were used for this object. Then, 0.5 g of dried samples of each replication was ground and ashed at 550 ºC in a porcelain crucible for 6 h, separately. The each yielded white ash was taken up in 2 M hot HCl, filtered into a 50-ml volumetric flask, and finally made up to 50-ml with distilled water. Sodium (Na), potassium (K), calcium (Ca) and chlorine (Cl) were measured on three samples. Sodium and potassium were analyzed using a flame photometer. Calcium and chlorine were analyzed with atomic absorption spectrophotometer and titration method, respectively [8].

Experimental design: This experiment was done in a completely randomized design (CRD) with 6 treatments and 9 replications in each. Data were analyzed with MSTAT-C and differences among means of treatments were compared by LSD at 5% level of confidence.

RESULTS

Leaf number (LN): The highest leaf number was resulted from N, NS3 and NS4 treatments. The lowest rate of this variable was obtained by NS treatment that had significant difference with others. In comparison among calcium salts, better results were found by $CaSO_4$ than with $CaCl_2$. It was shown that the highest and lowest leaf area obtained by N and NS treatments, respectively (Table 1). Similar to leaf number results, $CaSO_4$ led to the higher leaf area compared with $CaCl_2$ (data not shown).

Runner number (RN): The highest runner number was found on control and NS3 treatments. Data showed that the lowest rate of this variable was obtained by NS treatment, which hadn't significant differences with NS1 and NS2 treatments (Table 1).

Electrolyte leakage (EL): Although there were not any significant differences among treatments on this variable, however, from numerical aspect, the highest and lowest amounts of electrolyte leakage were obtained by NS and N (control) treatments, respectively. With attention to this point of view, it was clear that increment of calcium salts concentration (from 5 to 10 mmol) resulted in higher electrolyte leakage (Table 1).

Flower production (FP): Results showed that NaCl stress significantly reduced flower production compared with control. Addition of NaCl and calcium salts to nutrient solution reduced flower formation compared with control (Table 1).

Table 1. Effects of NaCl and different forms of calcium on some variables

Treatment*	LN	RN	EL (%)	FP	FS (%)
N	21.3a	3.1a	0.41 a	20.6a	100.0a
NS	3.6c	1.1c	0.56 a	10.8c	37.5f
NS1	11.0b	1.3c	0.42 a	13.7b	68.6d
NS2	9.0b	1.3c	0.44 a	6.4e	43.7e
NS3	19.3a	3.0ab	0.45 a	5.3e	81.2c
NS4	20.6a	2.5b	0.50 a	8.2d	93.8b

LN: leaf number; RN: runner number; EL: electrolyte leakage; FP: flower production; FS: fruit set.
*Within each column, same letter indicates no significant difference between treatments at 5% levels of LSD.

Table 2. Effects of NaCl and different forms of calcium on some variables

Treatment*	PA	TN	CPR	SLA	SLW	RWC
N	0.03a	2.43c	15.18bc	175.2a	0.0057a	0.83a
NS	0.03a	2.61b	16.35ab	169.8a	0.0055a	0.83a
NS1	0.03a	2.28d	14.25c	191.9a	0.0052a	0.82a
NS2	0.03a	1.95e	12.16d	183.4a	0.0055a	0.80a
NS3	0.03a	2.61b	16.31ab	186.3a	0.0052a	0.83a
NS4	0.04a	2.74a	17.14a	187.7a	0.0052a	0.78a

PA: proline amount; TN: total nitrogen; CPR: crude protein rates; SLA: special leaf area; SLW: special leaf weight; RWC: relative water content
*Within each column, same letter indicates no significant difference between treatments at 5% levels of LSD.

Table 3. Effects of NaCl and different forms of calcium on some variables

Treatment*	Shoot F.W. (g)	Root F.W. (g)	Shoot/root F.W.	TY (g)
N	226.2a	174.7a	1.2b	723.3a
NS	60.7f	61.5e	0.9d	220.9f
NS1	88.0d	75.6d	1.1c	461.0d
NS2	72.7e	91.5b	0.7e	280.2e
NS3	121.3b	81.3c	1.4a	479.8c
NS4	93.9c	60.2e	1.5a	683.0b

F.W.: fresh weight; TY: total yield

*Within each column, same letter indicates no significant difference between treatments at 5% levels of LSD.

Table 4. Effects of NaCl and different forms of calcium on some elements of root

Treatments*	Sodium (mgg^{-1} DW)	Potassium (mgg^{-1} DW)	Chlorine (%)	Calcium (gKg^{-1}DW)
N	7.0b	5.24a	19.64e	4.48d
NS	11.0a	5.78a	29.94d	3.72f
NS1	10.4a	5.33a	31.71cd	4.76c
NS2	12.5a	6.28a	34.32bc	5.00b
NS3	11.8a	5.29a	36.80b	3.95e
NS4	11.5a	5.19a	44.37a	5.80a

D.W.: Dry weight

*Within each column, same letter indicates no significant difference between treatments at 5% levels of LSD.

Table 5. Effects of NaCl and different forms of calcium on some elements of shoot

Treatments*	Sodium (mgg^{-1} DW)	Potassium (mgg^{-1} DW)	Chlorine (%)	Calcium (gKg^{-1}DW)
N	3.66e	19.98c	26.98d	4.76b
NS	10.45b	28.02a	34.67c	4.82a
NS1	11.93a	17.33c	41.06b	4.72bc
NS2	10.75ab	16.54c	42.72b	4.84a
NS3	7.24d	24.15b	98.10a	4.68c
NS4	8.83c	25.04ab	41.65b	4.70c

D.W.: dry weight

*Within each column, same letter indicates no significant difference between treatments at 5% levels of LSD.

Fruit set (FS): The highest and lowest percent of fruit set were resulted from N and NS treatments, respectively. Data showed that supplementary calcium significantly increased fruit set under NaCl stress.

Moreover, increment of $CaCl_2$ and $CaSO_4$ salts concentration, respectively reduced and increased fruit set (Table 1).

Proline amount (PA), total nitrogen (TN) and crude protein rates (CPR): There were not significant differences among treatments in proline amount of leaves.

The highest and lowest total nitrogen was resulted from NS4 and NS2 treatments, respectively. Data showed that only NaCl salt led to higher rate of this variable compared with control. Increment of concentration of $CaCl_2$ and $CaSO_4$ salts respectively, reduced and increased TN. Compared with control, results indicated the highest rate of crude protein rates by NS4 treatment, which had not significant differences with NS and NS3 treatment. Similar to TN results, increment of $CaCl_2$ salts concentration reduced CPR (Table 2).

Special leaf area (SLA), special leaf weight (SLW) and relative water content (RWC): There were not significant differences among treatments in SLA, SLW and RWC (Table 2).

Total yield (TY): NaCl stress significantly reduced total yield compared with control. Applied calcium salts

under NaCl stress improved this variable compared with NS treatment. Increment of $CaCl_2$ and $CaSO_4$ salts concentration from 5 to 10 mmol respectively reduced and increased this variable (Table 3).

Biomass production (BP): The highest and lowest shoot fresh weights (Sh.F.W.) were obtained in N and NS treatments, respectively. Application of different calcium salts increased this variable compared with NS treatment; however, increment of their concentration from 5 to 10 mmol reduced it. Data showed that NaCl salt stress (NS treatment) decreased root fresh weight (R.F.W.) in compare to control (N treatment). Application of calcium salts in lower concentration (5 mmol) significantly increased root fresh weight compared with NS treatment, although increment of $CaCl_2$ and $CaSO_4$ concentrations (from 5 to 10 mmol) increased and reduced this variable compared with lower concentration, respectively. The lowest and highest shoot/ root fresh weight were obtained by NS2, NS3 and NS4 respectively. Results showed that NS treatment significantly declined this ratio compared with N treatment (Table 3).

Nutrient analysis (NA): Results showed that applied NaCl salt significantly increased the Na^+ contents of root compared with control. There were not significant differences on this variable under NaCl and calcium treatments. Potassium content of root was not affected by different treatments, in compare to control. As data shown, compared with control, NaCl stress increased Cl^- content of root. Similar to Na^+ results in root, calcium forms induced the higher chlorine accumulation in this part compared with NS treatment. Moreover, as calcium salt concentration increased, Cl^- content of root increased (Table 4). The highest and lowest amounts of Ca^{2+} were found in roots of NS4 and NS treatments, respectively. Results showed that increment of calcium salts concentration (from 5 to 10 mmol) increased Ca^{2+} accumulation in root part of plants (Table 4). The highest and lowest sodium contents were found in shoot of NS1 and N treatments, respectively (Table 5). NaCl salt increased Na^+ accumulation in shoots. In comparison between two forms of calcium, $CaSO_4$ led to lower accumulated sodium in shoots (Table 5). Results indicated that NaCl stress increased the potassium contents of shoot, compared with control (N). Under different treatments of calcium, $CaSO_4$ resulted in higher accumulated potassium in shoot (Table 5). The highest and lowest amounts of chlorine were resulted from NS3 and N treatments, respectively. NaCl salinity significantly increased Cl^- content of shoots compared with unsalinized plants (Table 5). In comparison between control and NS treatments, NS led to higher amount of calcium in shoot (Table 5).

DISCUSSION

Sodium salts may affect the plants by 1) decreasing the water potential of the organs, 2) specific-ion toxicity and 3) changing solute transportation [13]. Schwarz [25] found that strawberry plants are sensitive to NaCl salinity. It is clear that calcium mitigates the harmful effects of NaCl stress [15], protecting the cell membrane from adverse effects of NaCl [7]. Munns and Termeat [21] reported that the reduction in leaf growth is the earliest response of glycophytes to salinity stress, which was related to low photosynthetic area. The lowest leaf number was found in NaCl (35 mmol) treatment, which not only correlated to the inhibitory effects of NaCl, but also correlated to the plants defoliation. This result was in agreement with findings of Saied et al. [24] in strawberry plants. In comparison among NS and calcium treatments, applied Ca^{2+} ameliorated the negative effects of NaCl on leaf area that was in agreement with Omami [22]. Increment of calcium concentration (from 5 to 10 mmol) decreased leaf number. NaCl salinity increased electrolyte leakage that was in agreement with Kaya et al. [15]. We suggested that because the plants faced to higher salts concentration, their cell membrane structure and disturbed its activity, thus higher rates of elements flowed into plant organs. Because of higher transpiration rate and gas exchange activity in leaves, flowing of elements is intensified into this organ, which cleared in nutritional analysis (Tables 4 & 5). As data shown, salinity significantly reduced flower production and fruit set that was in agreement with Awang and Atherton [3] on strawberry plants. Salinity reduced total yield of strawberry plants that was in agreement with Kaya et al. [16] on strawberry plants.

NaCl salt (NS treatment) caused an increment of concentration of some elements in shoots (Table 5) that was in agreement with Kaya et al. (16) results. It is thought that under this condition, because of cell membrane modification, their concentration increased in shoot. It can be concluded that high NaCl salt concentration (35 mmol) modifies the gas exchange activities and ionic contents of plant part. On the other hand, application of calcium is recommended under NaCl stress to reduce harmful effects of this salt. Moreover, it is proposed to evaluate action of different calcium forms under different salinity conditions.

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