Physiological responses of NaCl stressed cowpea plants grown in nutrient solution supplemented with $CaCl_2$

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Pitiuba cowpea (V*igna unguiculata* (L.) Walp.) plants were grown in nutrient solution and kept in a greenhouse up to pre-flowering stage. They were subjected to four different treatments: nutrient solution; nutrient solution containing 75 mmol.L^{-1} NaCl; nutrient solution containing 75 mmol.L^{-1} NaCl and 5 mmol.L^{-1} $CaCl_2$; and nutrient solution containing 75 mmol.L^{-1} NaCl and 10 mmol.L^{-1} $CaCl_2$. Salt stress strongly inhibited plant growth, caused a disturbance in plant-water balance, and increased the total content of inorganic solutes in the different plant parts, due mainly to accumulation of Na^+ and Cl^-. It also increased leaf and root soluble carbohydrates, reduced soluble amino nitrogen both in root tips and in the youngest trifoliate leaves, and reduced proline levels in root tips. Although the addition of $CaCl_2$ to the root environment of salt stressed plants caused a reduction in Na^+ content, specially in roots, it did not ameliorate the salt stress effects on plant-water relations and growth. Therefore, the results obtained do not support the hypothesis that supplemental calcium would ameliorate the inhibitory effects of NaCl-stress.

Key words: calcium, growth, salt stress, solutes accumulation, *Vigna unguiculata*, water relations.

Respostas fisiológicas de plantas de feijão-de-corda estressadas com NaCl e cultivadas em solução nutritiva suplementada com $CaCl_2$: Plantas de feijão-de-corda (*Vigna unguiculata* (L.) Walp.) Pitiúba foram cultivadas em solução nutritiva e conservadas em casa de vegetação até o estádio de pré-floração, e foram submetidas a quatro tratamentos, a saber: solução nutritiva; solução nutritiva contendo NaCl a 75 mmol.L^{-1}; solução nutritiva contendo NaCl a 75 mmol.L^{-1} e $CaCl_2$ a 5 e a 10 mmol.L^{-1}. O estresse salino inibiu fortemente o crescimento, provocou distúrbios no balanço hídrico das plantas e elevou o teor total de solutos inorgânicos nas diferentes partes das plantas, em vista, principalmente, do acúmulo de Na^+ e Cl^-. Além disso, aumentou o teor de carboidratos solúveis nas folhas e raízes, reduziu o teor de nitrogênio amino-solúvel, tanto nas extremidades das raízes como nas folhas trifolioladas mais jovens, e diminuiu os níveis de prolina nas extremidades das raízes. Embora a adição de $CaCl_2$ ao ambiente radicular de plantas estressadas tenha provocado redução no teor de Na^+, especialmente nas raízes, não minorou os efeitos do estresse nas relações hídricas e no crescimento das plantas. Os resultados obtidos, portanto, não dão suporte à hipótese de que o cálcio suplementar minoraria os efeitos inibitórios do estresse induzido por NaCl.

Palavras-chave: cálcio, crescimento, estresse salino, acúmulo de solutos, *Vigna unguiculata* (L.) Walp., relações hídricas.

INTRODUCTION

The addition of supplemental Ca^{2+} to the root environment has been suggested as a mean of enhancing plant tolerance to salt stress (Rengel, 1992; Epstein, 1998). Under salt stress conditions there is a decrease in the calcium/sodium ratio in the root environment which affects membrane properties, due to displacement of membrane-associated Ca^{2+} by Na^+, leading to a disruption of membrane integrity and selectivity (Cramer et al., 1985; Kinraide, 1998). This favors the increase of Na^+ inside the cells that could: change enzyme activity resulting in cell metabolical alterations; disturbance in K^+ uptake and partitioning in the cells and throughout the plant, that may even affect stomatal opening (Epstein, 1998). Therefore, impairing the ability of the plant to growth. The

addition of Ca^{2+} to the root environment of salt stressed plants maintains or enhances the selective absorption of potassium at high sodium concentrations, that will prevent the deleterious effects of the excess of Na^+ (Epstein, 1998). Another role attributed to supplemental Ca^{2+} addition is its help in osmotic adjustment and growth via the enhancement of compatible organic solutes accumulation (Colmer et al., 1996; Girija et al., 2002).

The mechanism of action of Ca^{2+}-enhanced tolerance of NaCl-stressed plants has been studied both at a cell-tissue-organ and at a molecular level, using *in vitro* cell cultures and *Arabidopsis thaliana* mutants (Liu and Zhu, 1997). However, there is not a consensus about the effectiveness of the use of supplemental Ca^{2+} for the enhancement of plant salt tolerance specially when applied as $CaCl_2$. While some authors have obtained results that favor its protective role on growth of salt stressed plants (Rengel, 1992; Song and Fujiyama, 1996), others have observed conflicting results (Lacerda et al., 1995; Kinraide, 1999; Reid and Smith, 2000). Therefore, in this paper the effects of supplemental $CaCl_2$ on growth, inorganic and organic solutes composition and plant-water relations were studied in NaCl-stressed cowpea plants aiming to clarify this discrepancy.

MATERIAL AND METHODS

Seed germination and growth conditions: Pitiuba cowpea (*Vigna unguiculata* (L.) Walp.) seeds were germinated on paper towels under laboratory conditions as described previously (Prisco and Vieira, 1976). Five days after planting seedlings were selected and transferred to plastic trays containing diluted (1/4 strength) and aerated Hoagland nutrient solution, and placed in a greenhouse. The seedlings were grown under these conditions for 5 days and then were transferred to 5.5 L plastic pots containing aerated half strength Hoagland nutrient solution. Salt additions into the nutrient solution (25 $mmol.L^{-1}$ NaCl and/or 5 $mmol.L^{-1}$ $CaCl_2$ per addition) were made every other day starting 24 h after the plants were transferred to the plastic pots. After these additions it were established 4 treatments: nutrient solution (C); nutrient solution containing 75 $mmol.L^{-1}$ NaCl (S); nutrient solution containing 75 $mmol.L^{-1}$ NaCl and 5 $mmol.L^{-1}$ $CaCl_2$ (SCa1); and nutrient solution containing 75 $mmol.L^{-1}$ NaCl and 10 $mmol.L^{-1}$ $CaCl_2$ (SCa2). At the last salt addition the strength of nutrient solution changed from half to full. Nutrient solution level was maintained constant throughout the experimental period by adding distilled water, and its pH was kept at 5.5 by adding 0.1 $mol.L^{-1}$ KOH or HCl. Changes in nutrient solution were made every 5 days, and each time the amount of salt added was restored to each pot in the salt stressed treatments. During the experimental period photosynthetically active radiation varied from a minimum of 120 at sunset to a maximal of 1,600 $\mu mol.m^{-2}.s^{-1}$ at midday, and mean air temperature and relative humidity inside the greenhouse were 31.1°C and 66.8 %, respectively.

Growth measurements: At the twenty-first day from the start of salt additions four plants from each treatment were harvested. Each plant was divided into leaf blades, stem and petioles, and roots. After determining leaf area using a Li-Cor area meter LI-3000 (Li-Cor., Inc., Lincoln, Nebraska, USA) and the fresh mass of the different parts they were dried in a forced air circulation oven at 60°C for 72 h.

Measurement of plant-water relations: The fifth trifoliate starting from the youngest leaf was used for water potential (Ψ_w) determination. The measurements were made in 4 different plants from each treatment at predawn of the last day of the experimental period using a pressure chamber (Soilmoisture Equipment Corp., Santa Barbara, CA, USA). Stomatal conductance (g_s) and transpiration rate (E) were determined along different days of the experimental period using a LI-1600 Steady State Porometer (Li-Cor., Inc., Lincoln, Nebraska, USA). The measurements were made on the central leaflet of the third trifoliate starting from the youngest leaf of four different plants from each treatment. Total water transpired by the plants during the experimental period was determined for four different plants from each treatment by calculating the amount of distilled water used to maintain the nutrient solution level in each pot. Transpiration ratio (mass of water consumed for the production of 1 gram of plant dry mass) and leaf succulence (leaf fresh mass/leaf area) were also determined.

Determinations of inorganic and organic solutes: Samples of 500 mg of oven-dried and ground material were ashed at 550°C for 4 h, and weighed for determination the % of ash in the oven-dried material. The ashed material was dissolved in 2 mL of 6 $mol.L^{-1}$ HCl, filtered into a volumetric flask, and the volume completed to 50 mL of demineralized water. This extract was used for Na, K, Ca, Mg and P determinations (Malavolta et al., 1989). Chloride was determined by titration after samples of 50 mg of oven-dried and ground material were extracted in 25 mL of demineralized water for 30 min

(Malavolta et al., 1989). Root tips (1 cm) and the youngest trifoliate leaves, after being frozen, were lyophilized, ground and samples of this material were used for organic solutes extraction and determinations. Samples of 2 mg of the freeze-dried material were suspended in 10 mL of distilled water, centrifuged at 3,000 g for 5 min, and the supernatant used for soluble carbohydrates determination (Dubois et al., 1956). Proline was extracted and determined according to Bates et al. (1973). Soluble amino nitrogen was extracted according to Ainouz (1970) and determined according to Cocking and Yemm (1954).

Experimental design and statistical analysis: The experimental design was completely randomized with four replicates per treatment. The data were subjected to analysis of variance, and the means were compared by Tukey's test ($p \leq 0.05$).

RESULTS

Plant growth: Plant growth measured both on dry mass and on leaf area (table 1) basis was strongly inhibited by salt stress. Total plant biomass production suffered a mean reduction of 60.4%, being the reduction of shoot growth (61.8 %) greater than that of root growth (47.6 %). As a result of this there was a decrease in shoot/root ratio of about 38 % (data not shown), typical of salt and water stressed plants. The addition of $CaCl_2$ (SCa1 and SCa2) to the nutrient solution containing 75 mmol.L^{-1} NaCl did not overcome the inhibitory effect of salt stress (S). Leaf growth was also strongly inhibited (66.2 %) by salt stress, and the addition of $CaCl_2$ to the nutrient solution containing 75 mmol.L^{-1} NaCl did not overcome this effect. On the contrary, the addition of 10 mmol.L^{-1} $CaCl_2$ enhanced leaf area inhibition from 66.2 to 77.8 %.

Plant-water relations: The data on plant-water relations are shown in table 2 and figure 1. Salt stress caused a reduction of about 37 % in leaf water potential (Ψ_w), and this reduction was not reversed by the addition of 5 or 10 mmol.L^{-1} $CaCl_2$ to the nutrient solution containing 75 mmol.L^{-1} NaCl. Salt stress reduced total water transpired during the experimental period by about 68 %. This reduction was not altered when plants were grown in nutrient solution containing 75 mmol.L^{-1} NaCl and 5 mmol.L^{-1} $CaCl_2$ (SCa1). However, the addition of 10 mmol.L^{-1} $CaCl_2$ to the nutrient solution containing 75 mmol.L^{-1} NaCl (SCa2) caused a greater decrease in water transpired. Transpiration ratio was also reduced (about 13 %) by salt stress, and the addition of 5 or 10 mmol.L^{-1} $CaCl_2$ to the nutrient solution containing 75 mmol.L^{-1} NaCl did not reverse this reduction. When leaf succulence was analyzed it was observed that it increased in about 30 % in the salt stressed plants (S), and the addition of 5 or 10 mmol.L^{-1} $CaCl_2$ to the nutrient solution containing 75 mmol.L^{-1} NaCl did not overcome this effect.

The daily variations of stomatal conductance (g_s) and transpiration rate (E) have demonstrated that the curves for control plants (C), salt stress (S), salt stressed containing 5 (SCa1) or 10 mmol.L^{-1} $CaCl_2$ (SCa2) showed the same general tendency (figure 1), that is, lower values in the morning tending to increase toward midday and decreasing toward the end of the afternoon. However, the rate values obtained at each hour of the day have shown that salt stress decreased both g_s and E, and that this decrease is greater in the treatments SCa1 and SCa2. None of the treatments showed a midday drop in transpiration, and the maximal transpiration rate occurred between 11:00 and 13:00 h for all treatments.

Inorganic and organic solutes: Although salt stress caused an increase in total inorganic solutes (Ash) in the different plant parts, this was not observed for all ions analyzed (table 3). Salt stress induced an increase in ash content in roots, stem + petioles, and leaves resulting mainly from Na^+ and

Table 1. Dry mass (DM) production and leaf area of cowpea plants grown in nutrient solution (C), in nutrient solution containing 75 mmol.L^{-1} NaCl (S), and in nutrient solution containing 75 mmol.L^{-1} NaCl and 5 mmol.L^{-1} (SCa1) or 10 mmol.L^{-1} (SCa2) $CaCl_2$.

Treatments	Dry Mass (g) [a]			Leaf area (cm^2)
	Root	Shoot	Total	
C	3.93 a	36.95 a	40.88 a	8,532 a
S	2.06 b (47.6)	14.12 b (61.8)	16.18 b (60.4)	2,880 b (66.2)
SCa1	1.51 c (61.5)	13.28 b (64.1)	14.79 b (63.8)	2,698 b (68.4)
SCa2	0.97 d (75.3)	7.82 c (78.8)	8.79 c (78.5)	1,893 c (77.8)

[a] Values followed by the same letter within the same column are not statistically different at $p \leq 0.05$ (Tukey test); values between parenthesis represent the percentage of reduction in relation to control treatment.

Cl^- accumulation. It induced increases in root Na^+ (930 %) and Cl^- (196 %), and decreases in all other ions studied. There were increases in Na^+ (880 %), Cl^- (576 %) and phosphorus (25 %) in stem + petiole, a decrease in K^+ (19 %), and the contents of Ca^{2+} and Mg^{2+} were not affected. Salt stress (S) also caused increases in leaf Na^+ (233 %), Cl^- (117%), phosphorus (55.5 %) and K^+ (19.3 %), and at the same time induced decreases in Ca^{2+} (23.8 %) and Mg^{2+} (15 %). The addition of 5 or 10 mmol.L^{-1} $CaCl_2$ to the nutrient solution containing 75 mmol.L^{-1} NaCl caused enhancement in total inorganic solutes (Ash) in all plant parts. This could be due basically to the accumulation of Cl^- in all plant parts and of Ca^{2+} in the shoot (stem + petiole + leaves). The additions of $CaCl_2$ to the nutrient solution of salt stressed plants induced a decrease in Na^+ content, specially in roots. However, this decrease was not sufficient to reach the Na^+ content of the control treatment (C) in any of the plant parts. It was also observed that the $CaCl_2$ additions (SCa1 and SCa2) were not sufficient to alter the K^+ decrease in roots and stem + petiole induced by salt stress, although in the leaves SCa2 brought K^+ content to the control treatment (C) level. As expected, the $CaCl_2$ additions partially overcame the salt stressed induced decreases in Ca^{2+} and Mg^{2+}, specially in the roots, but did not have any effect on salt induced changes in phosphorus contents.

Salt stress caused a significant increase in soluble carbohydrates both in roots (37.1 %) and in leaves (57.4 %), decreases in soluble amino nitrogen in roots (42.8 %) and in leaves (17.8 %), and also in root-proline (21.7 %), but leaf-proline content was not affected (table 4). Although the additions of $CaCl_2$ to the nutrient solution of salt stressed plants brought the root-carbohydrates content to the control treatment (C) level, the same did not happen with leaf-carbohydrates, i.e. they caused decreases in carbohydrates content, which did not reach the control treatment level. $CaCl_2$ additions did not reverse the salt stressed induced decrease in both root and leaf soluble amino-N nor in root-proline content. Finally, calcium additions contributed to an increase in leaf-proline content.

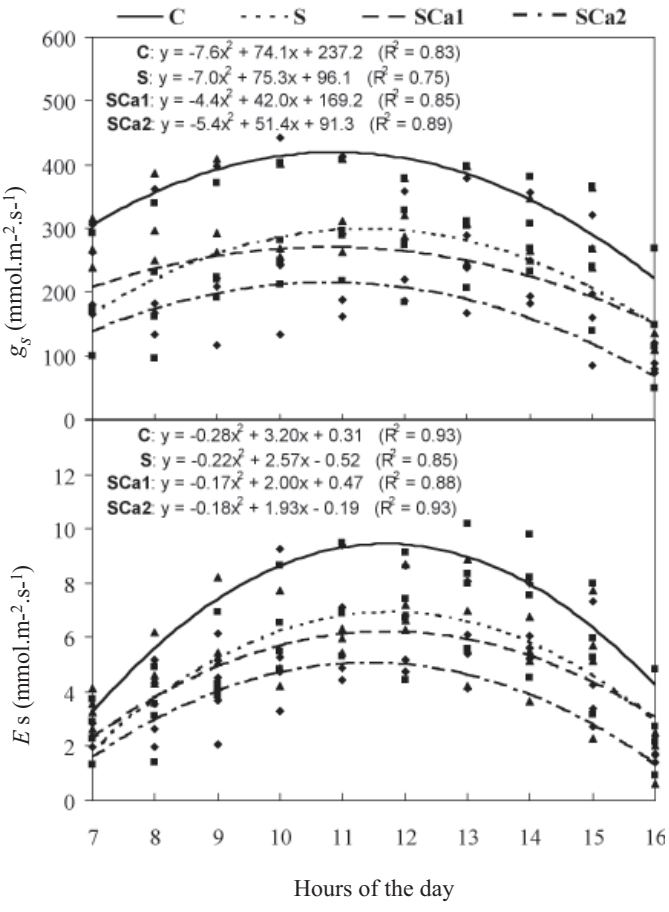

Figure 1. Stomatal conductance (g_s) and transpiration (E) of cowpea plants grown in nutrient solution (C), in nutrient solution containing 75 mmol.L^{-1} NaCl (S), and in nutrient solution containing 75 mmol.L^{-1} NaCl and 5 mmol.L^{-1} (SCa1) or 10 mmol.L^{-1} (SCa2) $CaCl_2$, at 9 (◆), 15 (■) and 21 (▲) days after starting NaCl additions.

Table 2. Water potential (Ψ_w), total water transpired (WT), transpiration ratio (TR) and leaf succulence (LFM/LA) of cowpea plants grown in nutrient solution (C), in nutrient solution containing 75 mmol.L^{-1} NaCl (S), and in nutrient solution containing 75 mmol.L^{-1} NaCl and 5 mmol.L^{-1} (SCa1) or 10 mmol.L^{-1} (SCa2) $CaCl_2$.

Treatments	Ψ_w (MPa) [a]	WT (L)	TR (g H_2O.g^{-1} DM)	LFM/LA (mg.cm^{-2})
C	- 0.19 a	14.71 a	398.21 a	19.56 b
S	- 0.26 b	4.76 b	344.95 b	25.45 a
Sca1	- 0.28 b	4.39 b	330.94 b	25.21 a
Sca2	- 0.29 b	2.85 c	343.28 b	25.59 a

[a] LFM = leaf fresh mass; LA = leaf area; values followed by the same letter within the same column are not statistically different at $p \leq 0.05$ (Tukey test).

Table 3. Inorganic solutes contents of cowpea plants grown in nutrient solution (C), in nutrient solution containing 75 mmol.L^{-1} NaCl (S), and in nutrient solution containing 75 mmol.L^{-1} NaCl and 5 mmol.L^{-1} (SCa1) or 10 mmol.L^{-1} (SCa2) $CaCl_2$.

Treatment	Solutes (mol.kg^{-1} DM) [a]						Ash (%)
	Na	Ca	Cl	K	P	Mg	
				Roots			
C	0.100 c	0.183 a	0.374 c	1.934 a	0.181 a	0.058 a	11.11 c
S	1.030 a	0.125 c	1.109 b	1.110 b	0.119 b	0.038 c	15.40 b
SCa1	0.696 b	0.148 b	1.017 b	1.207 b	0.116 b	0.046 b	17.41 ab
SCa2	0.661 b	0.155 b	1.340 a	1.294 b	0.113 b	0.050 ab	18.63 a
				Stem + Petioles			
C	0.087 c	0.293 b	0.143 c	1.376 a	0.039 c	0.054 a	13.43 d
S	0.852 a	0.265 b	0.966 b	1.115 b	0.048 b	0.054 a	14.73 c
SCa1	0.700 b	0.385 a	1.089 b	1.146 b	0.052 ab	0.054 a	15.76 b
SCa2	0.717 b	0.400 a	1.303 a	1.069 b	0.055 a	0.054 a	17.01 a
				Leaves			
C	0.052 c	0.653 c	0.394 c	1.061 c	0.058 b	0.083 a	12.92 c
S	0.174 a	0.498 d	0.857 b	1.266 a	0.090 a	0.071 b	14.82 b
SCa1	0.148 a	0.708 b	0.948 b	1.189 ab	0.084 a	0.063 c	15.78 b
SCa2	0.139 a	0.783 a	1.357 a	1.151 bc	0.090 a	0.063 c	17.44 a

[a] Values followed by the same letter within the same column and for each plant part are not statistically different at $p \leq 0.05$ (Tukey test).

DISCUSSION

Salt stress reduced plant growth, but contrary to what was observed by others (Epstein, 1998; Kaya et al., 2002), the addition of 5 mmol.L^{-1} $CaCl_2$ to the nutrient solution containing 75 mmol.L^{-1} NaCl did not ameliorate the salt stressed induced growth inhibition, and 10 mmol.L^{-1} $CaCl_2$ even increased growth inhibition (table 1). These discrepancies could be due to several factors, such as: use of different concentrations and sources of calcium salts, different NaCl concentrations (Reid and Smith, 2000; Munns, 2002), and environmental conditions under which the plants were grown (Yeo, 1999). The latter seems to be relevant because this same cowpea cultivar has responded differently to supplemental calcium, depending upon the evaporative demand of the environment under which they were grown (Lacerda et al., 1995). Another interesting aspect of the response of salt-stressed plants to supplemental calcium is the duration of the treatment. For instance, salt-stressed plants grown under high evaporative demand of the air and supplemented with calcium during their entire life cycle, despite having their vegetative growth inhibited, had their pod production ameliorated (Lacerda et al., 1995).

The beneficial effects of calcium additions to the root environment of NaCl-stressed plants are associated with the maintenance of cell membrane integrity, reducing Na^+ and favoring K^+ absorption in salt stressed plants (Epstein, 1998). Although the addition of calcium to the nutrient solution did not favor K^+ absorption in salt-stressed plants it reduced Na^+ absorption by the roots and contributed to its reduction in the shoot. On the other hand, Cl^- contents were the highest in plants subjected to treatment containing higher calcium concentration in nutrient solution (table 3). It should be also noted that leaf succulence in this treatment (SCa2) was similar to those of S and SCa1 (table 2) even though its Cl^- content was considerably higher. The fact that the Cl^- concentration, expressed on a water content basis (data not shown), was considerably higher in the SCa2 treatment could partially explain the greater growth reduction in this treatment, due to toxic effects of this ion (Zekri and Parsons, 1990). In addition to this, the total salt concentration in nutrient solution was the highest in this treatment (SCa2), which could have contributed to this higher growth reduction (Song and Fujiyama, 1996; Reid and Smith, 2000). Therefore, although there was a beneficial effect of $CaCl_2$ addition on the decrease of Na^+ accumulation in the shoot, this appeared to be counterbalanced by the deleterious effects of tissue Cl^- accumulation and by the lowering of the Ψ_w in the root environment, due to the increase in total salt concentration in the nutrient solution (Zekri and Parsons, 1990).

Table 4. Organic solutes contents of cowpea plants grown in nutrient solution (C), in nutrient solution containing 75 mmol.L^{-1} NaCl (S), and in nutrient solution containing 75 mmol.L^{-1} NaCl and 5 mmol.L^{-1} (SCa1) or 10 mmol.L^{-1} (SCa2) $CaCl_2$.

Treatments	Solutes (mol.kg^{-1} DM) [a]		
	Soluble carbohydrates	N-amino soluble	Proline
		Root tips	
C	0.398 b	0.553 a	0.012 a
S	0.546 a	0.316 b	0.009 b
Sca1	0.590 a	0.373 b	0.010 b
Sca2	0.405 b	0.348 b	0.009 b
		Youngest trifoliate leaf	
C	0.535 c	0.677 a	0.020 b
S	0.842 a	0.556 b	0.021 b
Sca1	0.709 b	0.412 c	0.024 a
Sca2	0.690 b	0.532 b	0.026 a

[a] Values followed by the same letter within the same column are not statistically different at $p \leq 0.05$ (Tukey test).

It was also suggested that the addition of calcium to the root environment of NaCl-stressed plants would help organic solute accumulation in the roots, which could contribute to root osmotic adjustment, favoring the maintenance of plant-water balance and growth (Colmer et al., 1996; Girija et al., 2002). Salt-stressed cowpea plants accumulated soluble carbohydrates as well as ions both in roots and in leaves. At the same time, proline and soluble amino-nitrogen were either not affected or decreased as a result of salt-stress (table 4). Therefore, the accumulation of ions and carbohydrates could be accounted for by the osmotic adjustment that could have contributed to the maintenance of their water balance under stress conditions (table 2). The addition of calcium to the nutrient solution of the salt stressed plants (SCa1 and SCa2 treatments) reduced soluble carbohydrate contents and did not alter or increased proline content, when compared to plants of the S treatment (table 4). The decrease in carbohydrates content could be explained by their deversion to other anabolic and catabolic pathways, and could account for the lower productive capacity as well as for the enhancement of mature leaf senescence observed by Munns (2002). The increase in leaf proline content as a result of calcium addition could be associated to leaf dehydration (Lacerda et al., 2003) or to a higher ion accumulation (Weimberg et al., 1984). Although proline content increased as a result of salt stress, its concentration was not high enough to explain their contribution to osmotic adjustment. The increase in proline content was more conspicuous in plants that showed the greatest growth reduction, reinforcing the idea that increase in proline concentration is the result of stress damage and it is not associated with stress tolerance (Ferreira et al., 1979; Lacerda et al., 2003). Proline content in root tips did not differ between S and SCa1 or SCa2 treatments, and they were lower in salt-stressed than in the control treatment (table 4). These results do not support the hypothesis that calcium promotes organic solute accumulation, mainly proline, in root tips favoring osmotic adjustment and root growth under salt stress conditions (Franco et al., 1999).

The reductions in stomatal conductance and in transpiration rates were, in general, higher in plants supplemented with calcium than in plants subjected only to NaCl stress (figure 1), these results being partially related to the increases in tissue ions accumulation (table 3) and plant growth reduction (table 1). Although these mechanisms could be beneficial because they reduce plant water loss, they also reduce net CO_2 assimilation and consequently plant productivity (Seemann and Critchley, 1985). So, the additions of $CaCl_2$, despite having some beneficial effects on decreasing Na^+ absorption, were not sufficient to maintain plant-water balance (figure 1) and ameliorate the growth of salt stressed plants.

Therefore, the results obtained reinforce the idea that the beneficial effects of calcium on growth of NaCl-stressed plants will only appear if they supplant the deleterious effects associated with the increase in total salt concentration of the root environment, i.e., osmotic effects resulting from the decrease in osmotic potential of the nutrient solution (Reid and Smith, 2000) and "toxic" effects due to tissue ions accumulation, especially those of the companion anion, such as Cl^- (Zekri and Parsons, 1990).

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