



Effects of NaCl-salinity on growth and inorganic solute accumulation in young cashew plants¹



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Abstract: The NaCl effects on the growth and inorganic solute accumulation were studied on 30-day-old cashew plants (*Anacardium occidentale* L.) hydroponically grown for 8 days (short term) and 40 days (long-term) with NaCl at different levels. The shoot fresh mass yielded after 40 days, in response to 50 and 100 mol m⁻³ NaCl, decreased by 25 and 75%, respectively. This decrease was markedly low in root fresh mass, which did not change under 50 mol m⁻³ NaCl and decreased nearly to 30% under 100 mol m⁻³ NaCl, as compared to control plants. In short-term experiment, salinity induced only slight changes of K⁺ tissue concentrations in the whole plant. In the long-term experiment, K⁺ tissue concentrations were substantially decreased, particularly in roots. In response to time and increasing levels of salinity, Na⁺ and Cl⁻ ions concentrations reached toxic levels in leaves. Thus, cashew plants already from the 4th day of salinity stress exhibited earlier symptoms of ionic toxicity, and therefore they were not able to regulate metabolic and physiological functions under these harmful conditions.

Key words: *Anacardium occidentale*, salinity stress, ionic toxicity, potassium, sodium, chloride

Efeitos da salinidade induzida por NaCl sobre o crescimento e acumulação de solutos inorgânicos em plantas jovens de cajueiro

Resumo: Os efeitos da salinidade sobre o crescimento e acumulação de solutos inorgânicos foram avaliados em plantas de cajueiro (*Anacardium occidentale* L.), com 30 dias de idade, cultivadas em diferentes doses de NaCl, por 8 dias (curta duração) e 40 dias (longa duração). A produção de massa fresca (MF) da parte aérea, após 40 dias, foi reduzida, aproximadamente, 25 e 75% sob 50 e 100 mol m⁻³ de NaCl, respectivamente. Nas raízes, a produção de MF não foi afetada em 50 mol m⁻³ de NaCl, entretanto decresceu 30% em 100 mol m⁻³ de NaCl. No experimento de curta duração, a concentração de K⁺ nos diferentes tecidos foi similar àquela das plantas controle, enquanto que, no experimento de longa duração, a concentração de K⁺ foi fortemente reduzida, principalmente nas raízes. Em resposta ao tempo e a salinidade crescente, as concentrações de Na⁺ e Cl⁻ atingiram níveis tóxicos nas folhas o que levou, a partir do quarto dia do estresse salino, ao surgimento de sintomas típicos de toxicidade por estes íons. Em consequência, as plantas de cajueiro não foram hábeis em regular suas funções metabólicas e fisiológicas nessas condições adversas de crescimento.

Palavras-chave: *Anacardium occidentale*, estresse salino, toxicidade iônica, potássio, sódio, cloreto

INTRODUCTION

Under natural conditions, crops are often exposed to several environmental stress conditions (mainly salinity stress) that decrease production. At the whole-plant level, the effect of stress is usually perceived as a decrease in photosynthesis and growth and is associated with alterations in metabolism

(Ormatex et al., 1998). At the molecular level, the negative effect of stress on plants may be, in part, a consequence of the ionic toxicity damage to important molecules.

Salinity affects production of agricultural crops world wide (Morales et al., 1992), including areas in northeastern Brazil, salinity being a major factor limiting crop productivity (Ortiz et al., 1994). Salinity appears to affect two plant processes: water

and ionic relations (Cramer & Novak, 1992). Thus, the main problem for a plant upon NaCl exposure is that, on the one hand, since external osmotic potential is much lower than in normal soils, compatible solutes must be accumulated to high levels to create a water potential gradient osmotic adjustment to facilitate inward water movement. On the other hand, increasing concentrations of Na⁺ and Cl⁻ ions in the symplasm eventually may become toxic (Gouia et al., 1994) and decrease internal availability of nutrients arising from a lower uptake rate and the competition between nutrient ions, such as K⁺ and Na⁺ and Cl⁻ (Bottacin et al., 1984). Thus Na⁺ and Cl⁻ uptake, accumulation and osmoregulation are subject of intensive research for possible mechanisms of salt tolerance (Flowers et al., 1977).

The mechanism of Na⁺ influx across the plasma membrane is still unknown (Niu et al., 1995). It is thought that Na⁺ acts as a competitor of K⁺ uptake (Schroeder et al., 1994) which, in turn, suggests that the uptake mechanism for such cations is the same. Plant roots utilize two systems for K⁺ uptake (system 1 has K⁺ high affinity, μm ; and system 2 has lower K⁺ affinity, mm). The former allows uptake at low K⁺ concentration, but it is inhibited by Na⁺ (Rains & Epstein, 1967) and the later mediates uptake at high external K⁺ concentration had a less pronounced K⁺/Na⁺ selectivity. Long ago, it was suggested that Na⁺ influx into plants occurs via the low-affinity rather than the high-affinity K⁺ uptake system (Rains & Epstein, 1967). In addition, little is known on intracellular uptake and vacuolar accumulation of Cl⁻. Presumably, genotypes that are most adapted to salt regulated ion uptake across the plasma membrane at a rate at least compatible with the capacity for vacuolar compartmentation (Binzel et al., 1988). Finally, to gain a more thorough understanding of how salt stress mechanisms act in a woody plant, the aim of the present study was to investigate the possible role of specific ion accumulation on growth and K⁺ content in cashew plants grown upon short and long-terms and to determine the variation in effect of salinity in different plant parts. In addition, the response to increasing concentrations of salt on transpiration rates was also studied.

MATERIAL AND METHODS

Plant material and growth conditions

Cashew (*Anacardium occidentale* L. cv 1001 - a relatively salt sensitive species, Viégas & Silveira, 1999) seeds were surface-sterilized by rinsing with 5% (v/v) sodium hypochloride followed by thorough washing with distilled water. After 12 h imbibition (soaked in air bubbled distilled water in darkness), seeds were placed to germinate at a depth of 2.0 cm in plastic tanks (30.0 cm in long x 40.0 cm high) containing agricultural vermiculite irrigated daily (once) with solution loading CaSO₄ 1.0 mmol dm⁻³, until germination and emergence of a homogenous seedlings population had been achieved. Afterwards, 20-day-old seedlings were transferred to plastic pots filled with 1.0 dm³ of aerated one-half-strength Hoagland nutrient solution, for acclimatization. The pH of the nutrient solution was kept at 5.5 ± 0.5 range. The seedlings were acclimatized in the solution for 10 days, under green house conditions.

Experiment 1 (short term): The study was performed on 30 day-old cashew plants without addition of NaCl or by adding NaCl 100 mol m⁻³ to the full strength Hoagland nutrient solution continuously aerated. The experiment was carried out in plastic pots containing 1.0 dm³ of nutrient solution. Salt addition was made at the beginning of the light period (beginning of day). During the experiment nutrient solutions were fully changed daily and the pH kept in 5.5 ± 0.5 range. On 0, 1, 2, 4, and 8 days after start of experiment, 3 plants from each treatment were harvested.

Experiment 2 (long term): Salt treatments of 0, 50 and 100 mol m⁻³ NaCl full strength Hoagland nutrient solution were applied to 30-day-old cashew plants for 40 days. The experiment was carried out in plastic pots containing 5.0 dm³ of nutrient solution. The experimental conditions were similar to those for Experiment 1. During the experiment, nutrient solutions were changed fully twice a week and the pH kept at 5.5 ± 0.5 range. At the end of 40 days, 5 plants per treatment were harvested.

At harvests, in both experiments, nutrient solution containing sodium chloride was removed and, after rinsing the roots with distilled water, plants were cut off into root, stem, and leaf. Fresh mass was then determined and plant parts were immediately frozen in liquid nitrogen and stored at -80 °C, for later extraction and analysis of mineral composition.

Plants were grown under greenhouse condition under natural 12 h photoperiod and temperature varying from 28 to 35 °C during the day and from 24 to 27 °C during the night. Relative humidity was of about 40% during the day and 85% during the night. The experiments, which were randomized, had 3 (Experiment 1) and 5 (Experiment 2) independent replicates per treatment, each replicate consisting of 1 plant.

Mineral composition

Lyophilized plant tissue was powdered and subjected to wet digestion with (4:1) HNO₃: HClO₄ (Chapman & Pratt, 1961). The resulting solutions were properly diluted and analyzed for K⁺ and Na⁺ by flame photometer.

Cl⁻ was extracted from the lyophilized tissue with water and determined by titration against standard AgNO₃ using potassium chromate as indicator (Malavolta et al., 1989).

Plant transpiration and tissue water content

Estimates of whole plant transpiration (Experiment 1) were carried out through reduction of the volume of the nutrient solution daily in each pot (Reed & Hageman, 1980). Comparable pots, with nutrient solution but without plants, were used to correct for evaporation.

Root, stem and shoot water content was determined by weighing tissue before (fresh mass - FM) and after complete lyophilization (dry mass - DM) using an analytical balance (Coombs & Hall, 1982).

Data analysis

The effect of treatments on fresh mass yielded in the shoot and root was tested by ANOVA. Means were compared by LSD (least significant difference) at the 0.05 confidence level using Student's *t*-test. Standard errors are reported in the figures.

RESULTS AND DISCUSSION

Plant growth

After the use of NaCl at a substrate concentration of 100 mol m⁻³ (short-term experiment) and 50 and 100 mol m⁻³ (long-term experiment), to produce an osmotic shock to *Anacardium occidentale* plants, one must distinguish between early and late effects of salinity. In such experiment, osmotic shock and specific ion effects for Na⁺ and Cl⁻ ions are involved in plant physiological and biochemical responses related to plant grown under condition of salinity.

Growth responses of young cashew plants to time and increasing salinity levels, in the nutrient solution, are shown in Tables 1 and 2. At the end of short-term experiment (8th day), NaCl induced a reduction in shoot fresh mass approximately of 25% with respect to absence of salinity (control), whereas in root fresh mass significant differences were not found (Table 1). In the long-term experiment (40 days), increasing levels of salinity markedly reduced plant growth. The shoot fresh mass of plants treated with NaCl 50 mol m⁻³ was 75% of that of untreated plants, while root fresh mass remained at a level comparable to the control. In plants treated with 100 mol m⁻³ NaCl, shoot and root fresh mass were only 33 and 67%, respectively, of the control plants and therefore NaCl decreased shoot to root ratio (Table 2). Root to shoot ratio can be thought of as being governed by a functional balance between water uptake by the root and photosynthesis by the shoot. This functional balance is shifted if water supply decreases, which

Table 1. Effect¹ of NaCl over time (day) on shoot and root fresh mass (g plant⁻¹) of young *Anacardium occidentale* plants grown with NaCl 100 mol m⁻³

Treatment	Time (day)	Shoot ² (g plant ⁻¹)	Root ² (g plant ⁻¹)	Shoot/Root
Control	0	16.13 a	13.96 a	1.15
	1	17.04 a	13.42 a	1.27
	2	17.10 a	15.88 a	1.08
	4	18.31 a	15.55 a	1.18
	8	22.60 b	16.18 a	1.39
NaCl	0	16.13 a	13.96 a	1.15
	1	15.15 a	13.67 a	1.08
	2	15.07 a	15.41 a	0.98
	4	17.04 a	14.97 a	1.14
	8	17.05 a	15.09 a	1.13

¹ Means followed by the same letter in columns are not significantly different at $p = 0.05$, Student's *t*-test

² All values are mean of 3 independent samples. ANOVA results: NaCl^m(S); Time^m(T) and S x T^{ms} for shoot, NaCl^m(S); Time^m(T) and S x T^{ms} for root. (** indicate significant difference at $p < 0.05$; ns non-significant)

Table 2. Effect¹ of NaCl on shoot and root fresh mass (g plant⁻¹) of young *Anacardium occidentale* plants grown with 0, 50, and 100 mol m⁻³, over 40 days

NaCl (mol m ⁻³)	Shoot (g plant ⁻¹)	Root (g plant ⁻¹)	Shoot/Root
0	28.16a	21.07a	1.33
50	21.61b	21.47a	1.00
100	11.66c	14.12b	0.83

¹ Means followed by the same letter in columns are not significantly different at $p = 0.05$, Student's *t*-test

² All values are mean of 5 independent samples

is widely observed in non-halophytes grown under salinity condition. In addition, salt stress may reduce plant growth by causing ion toxicity, ion imbalance, or a combination of any of these adverse factors (Greenway & Munns, 1980). However, the exact mechanisms by which salinity inhibits shoot growth more than root growth are still poorly understood.

Cashew plants started to show visible signs of earlier senescence in leaf and reduction in leaf expansion from the 4th day of osmotic shock (data not shown). This could be the result of excess Na⁺ and Cl⁻ ions inducing toxicity on fully expanded leaves (Nabil & Coudert, 1995). Such injury is seen in many woody plants growing under salinity (Bernstein, 1975), and leads mainly to a decrease in photosynthetic leaf area. As a result, and in agreement with the findings of Munns & Termaat (1986), the production of carbohydrate declines and productivity falls below a level capable of sustaining further growth.

Although plant transpiration had substantially decreased by about 50% already during the initial few minutes of salinity treatment from 120 in control to 58 mL plant⁻¹ day⁻¹ in salt treated plants, the estimates of tissue water contents (near to 83% in stems, 85% in leaves and 93% in roots) did not differ between control and stressed plants in such experiments. Thus, and in agreement with Nabil & Coudert (1995) and Ludlow & Muchow (1990), this suggest that bulk tissue turgor was not limiting growth in this study and emphasize the possible implications of salinity effects linked to metabolic imbalance due certainly to Na⁺ and Cl⁻ excess as stated below. Presumably, cashew plants were not able to regulate Na⁺ and Cl⁻ uptake at a rate that is compatible with their capacity for vacuolar compartmentation. Thus, transport processes at the plasma membrane and tonoplast that regulate ion influx and efflux, particularly those involved in the control of Na⁺ uptake and vacuolar compartmentation are of crucial importance to salinity adaptation.

Inorganic solute accumulation

Saline treatment led to an accumulation of Cl⁻ and Na⁺ in all parts of the plants. The distribution of Cl⁻ and Na⁺ within the plants differed with time. After 24 h (1st day) of treatment (short-term experiment), the concentration of Cl⁻ and Na⁺ ions in leaf remained near unchanged irrespective of salinity, while in stem and root Cl⁻ and Na⁺, respectively, had a value about 2 and 20 fold higher than that of the untreated control plants (Figure 1, 2), preventing apparently harmful ions (Cl⁻ and Na⁺) from reaching the leaves in the earliest stage of salinity (1st day). However, the reverse occurred at the 2nd and 4th day of salinity treatment, when Na⁺ and Cl⁻ concentrations in leaf, respectively, were as great as that seen in the stem and root (Figure 1, 2) and, upon long-term experiment (40 days), Na⁺ and Cl⁻ accumulation in leaf reached an amount nearly 55 and 70% higher than in stem and root, respectively, in plants growing in NaCl 100 mol m⁻³ (Figure 3). It leads to the conclusion that this pattern for Cl⁻ and Na⁺ plant distribution point to a limited root/stem boundary as the place where Cl⁻ and Na⁺ absorption is controlled in cashew plants. Thus, in agreement with Cheeseman (1995), the physiological disturbances induced by NaCl in the present study were, certainly, associated with an accumulation of excessive concentrations of chloride and/or sodium ions in the leaves.

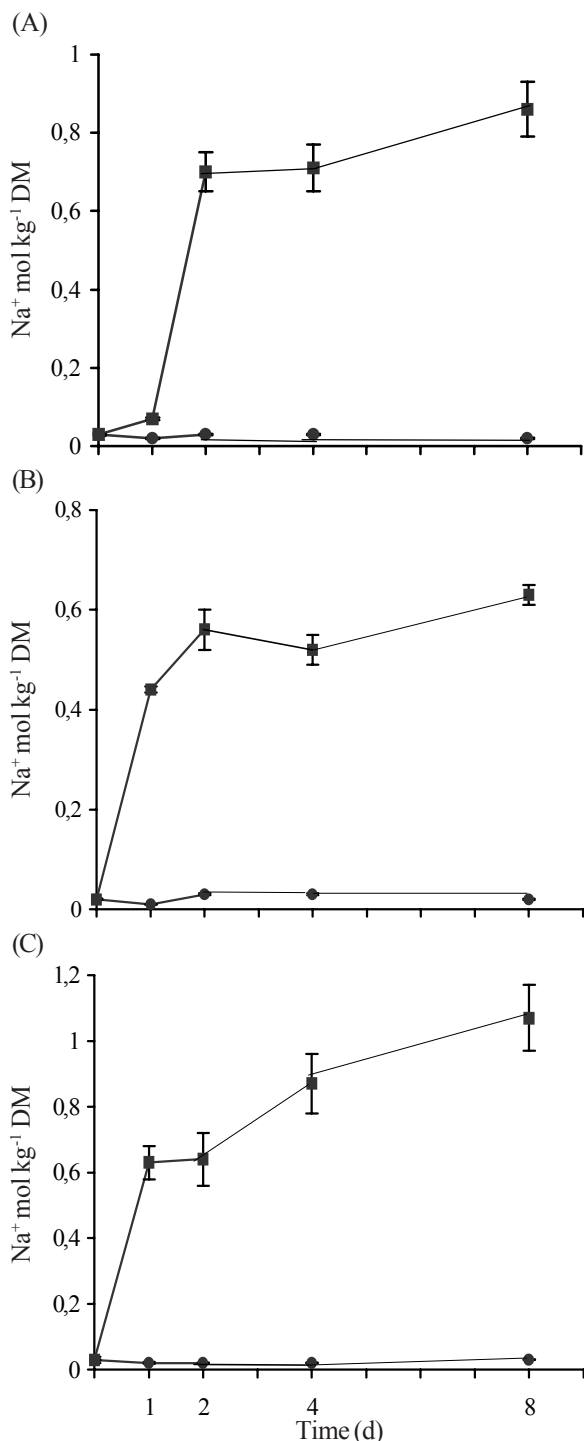


Figure 1. Accumulation of Na^+ in leaf (A), stem (B), and root (C) dry mass (DM) of hydroponically grown young *Anacardium occidentale* plants. ●, control nutrient solution; ■ nutrient solution salinized with 100 mol m^{-3}

A more detailed analysis of ion partition in all plant parts shows clearly that Cl^- tissue accumulation had significantly been increased after the 1st day of treatment, both in stem and root and after the 2nd day in leaf (Figure 2). It is interesting to explain that inside negative membrane potential across the plasma membrane that occurs when ion homeostasis is established (before NaCl shock), and maintained, is a substantial thermodynamic barrier to Cl^- influx, even at relatively high external concentration of these ions. If, however, Na^+ influx

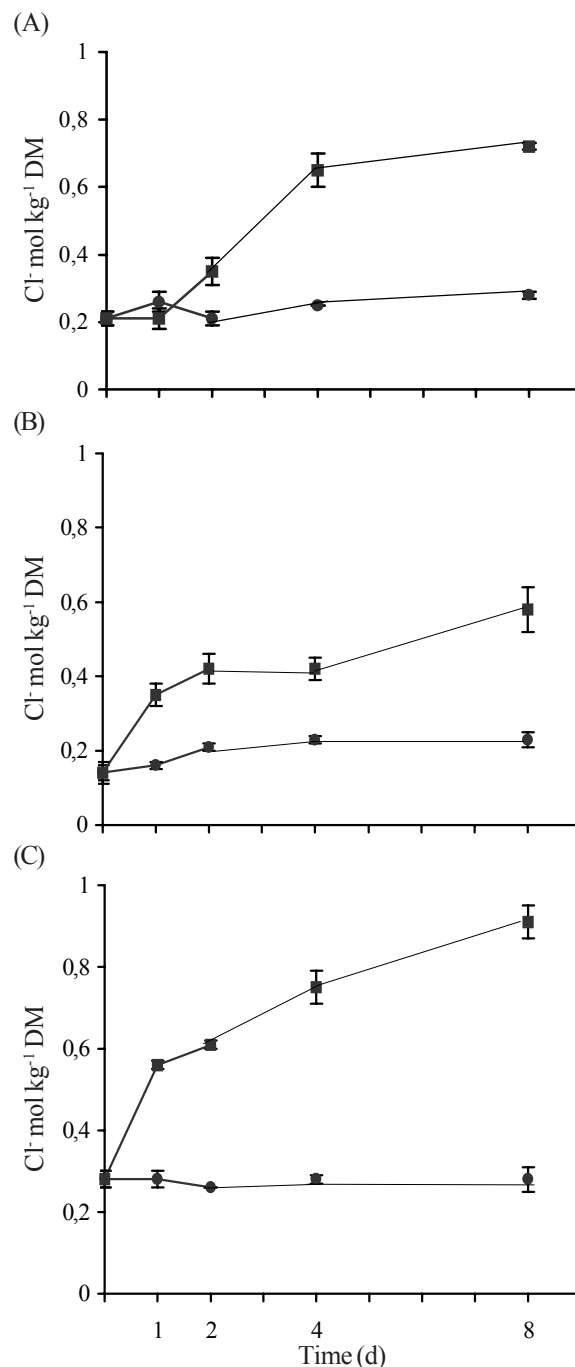


Figure 2. Accumulation of Cl^- in leaf (A), stem (B), and root (C) dry mass (DM) of young *Anacardium occidentale* plants hydroponically grown in $\text{NaCl } 100 \text{ mol m}^{-3}$. ●, control nutrient solution; ■ nutrient solution salinized with 100 mol m^{-3}

depolarizes $\Delta\Psi$ across the plasma membrane (after NaCl shock), the Cl^- can be taken up passively through an anion channel (Skerrett & Tyerman, 1994).

As is seen in Figure 4, tissue K^+ concentrations were nearly unchanged between control and salt treated plants along with short-term experiment (8 days). It has been observed that NaCl sensitive species grown on complete culture solution show a clear stimulation of K^+ uptake by NaCl (Cheeseman, 1995). In other study, however, NaCl was either without effect or inhibited K^+ uptake in a wide range of terrestrial species (Maathuis et al., 1996). However, in long-term experiment (40 days), at different

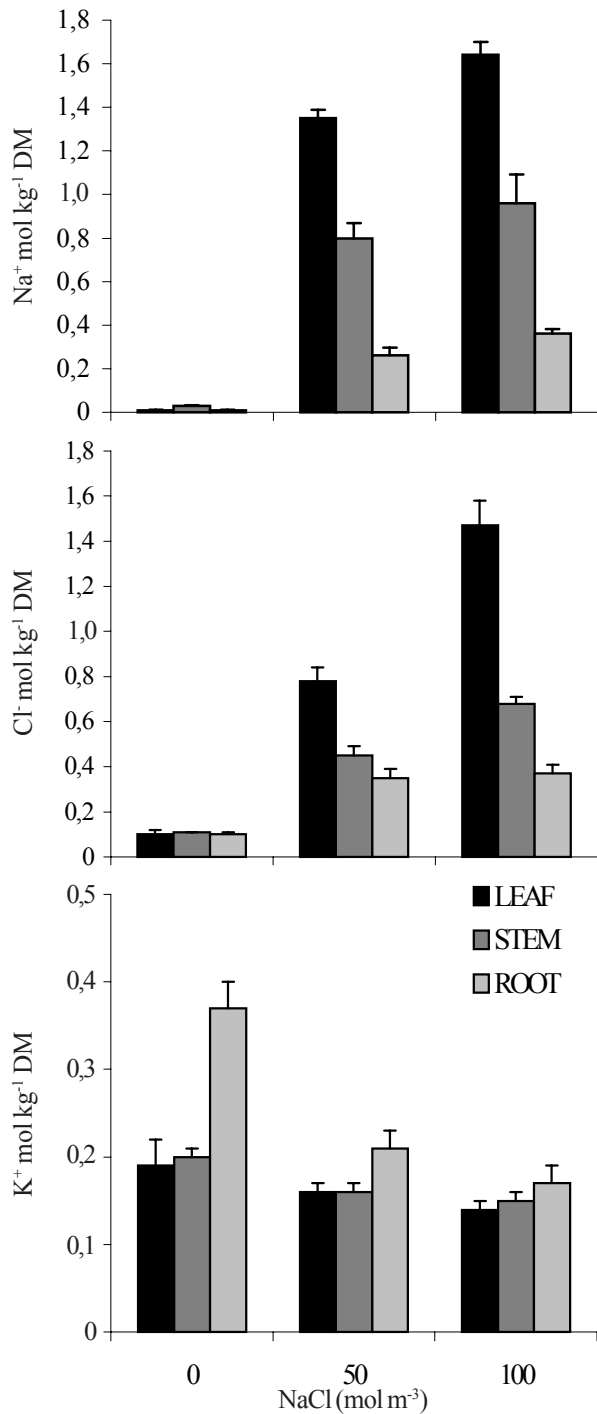


Figure 3. Accumulation of Na⁺, Cl⁻ and K⁺ in leaf, stem and root dry mass (DM) of young *Anacardium occidentale* plants hydroponically grown with NaCl 0, 50 and 100 mol m⁻³

levels of salinity, K⁺ tissue accumulations of cashew plants hydroponically grown were lower for salinized plants than for control in all plant parts, and the difference between control and plants was higher, particularly in root (around 50%), while leaf and stem K⁺ accumulations were decreased by about 27 and 25%, respectively, in the highest level of NaCl (Figure 3). Since K⁺ concentrations in leaf, stem, and root were not affected by NaCl along with short-term experiment (Figure 4), this decrease in K⁺ tissues accumulation in salinized plants upon long-term experiment, suggest root membranes may have become more permeable under long-term salinity. It is most

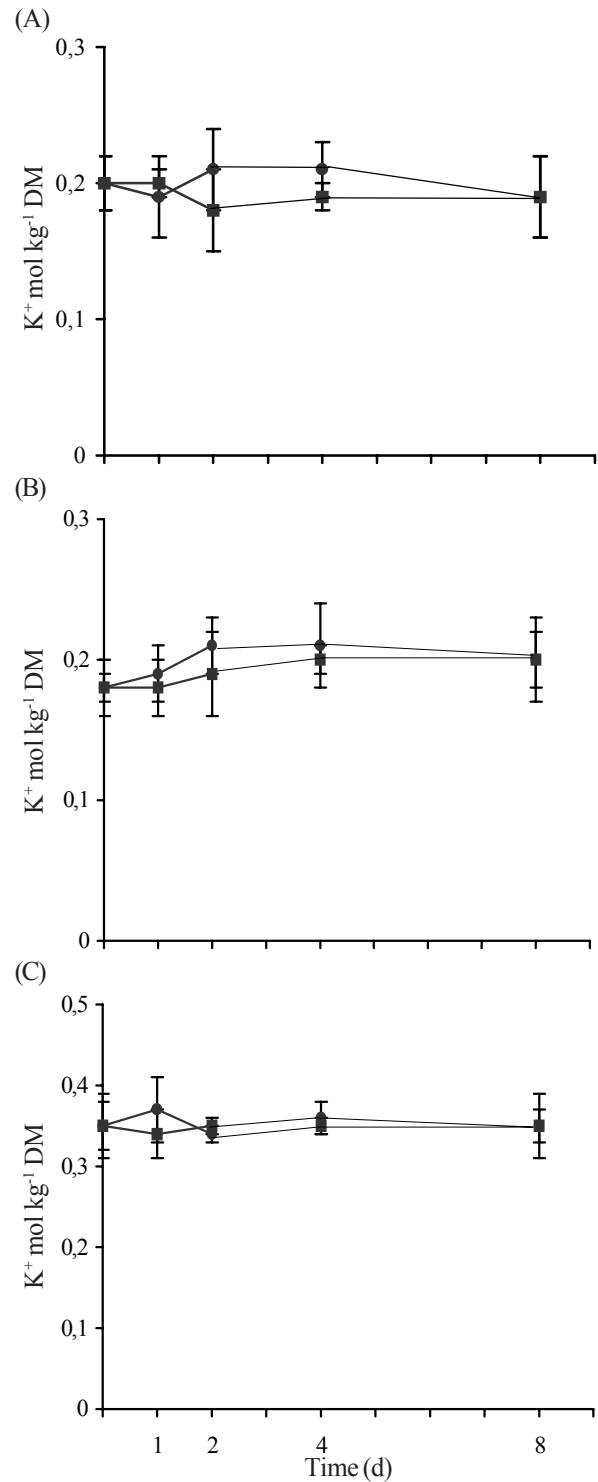


Figure 4. Accumulation of K⁺ in leaf (A), stem (B), and root (C) dry mass (DM) of young *Anacardium occidentale* plants hydroponically grown in NaCl 100 mol m⁻³. ●, control nutrient solution; ■ nutrient solution salinized with 100 mol m⁻³

likely that Na⁺ had displaced Ca²⁺ from the plasma membrane resulting in a change of plasma membrane permeability, that can be detected as decrease in K⁺ tissue concentration, particularly in root, or leakage of K⁺ to the external solution (Teleisnik & Grunberg, 1994; Lacan & Durand, 1995). Thus, the capacity to retain membrane integrity and ion selectivity under salinity is essential for maintaining internal homeostasis.

Indeed, data suggest that cashew plants, at least under short-term salinity stress, may have a higher capacity to retain K^+ under salinity. However, it seems still uncertain as to how it happens. Thus, studies aiming to determine this process could point out physiological targets for further breeding programs aimed at increasing the salt tolerance of crops, as K^+ is one of the traits linked to salinity stress tolerance in some plant species (García-Legaz et al., 1993) and the main inorganic osmoticum in plants, probably because it is energetically cheaper than organic metabolites (Ortiz et al., 1994). However, a major unresolved question is the extent and importance of both short and long term stress responses for sustained tolerance and their effects on agricultural desirable traits in crop plants (Winicov, 1998).

Furthermore, the remarkable and accumulation of Na^+ observed in leaves of cashew plant and the slight change in leaf K^+ content (Figure 1 A, 4 A), resulted in a decreased K^+/Na^+ ratio at the end of short-term experiment (8th day) from 9.5 in control to 0.2 in salt treated plants ($NaCl$ 100 mol m^{-3}). In addition, the plants grown during 40 days (long-term experiment) with $NaCl$ 100 mol m^{-3} exhibited K^+ to Na^+ ratio near to 0.01. K^+/Na^+ ratio higher than 1.0 has been considered as an plant index of adaptive response to salinity. Thus, Bottacin et al. (1984), showed that K^+/Na^+ ratio was 1.46, for the resistant, and 0.64, for the susceptible genotypes. They concluded that resistance is characterized by the ability to maintain a K^+/Na^+ ratio adequate for the metabolic requirements. The results of this study suggest that maintenance of K^+ tissue concentration despite enhancement of Na^+ accumulation along with experiments may be unfavorable for cashew plants grown in salt condition and therefore increased K^+/Na^+ selectivity of the K^+ uptake system might represent a significant adaptation to high concentrations of $NaCl$. At the whole plant level, it is generally accepted that increased K^+/Na^+ selectivity during uptake and reduced Na^+ translocation from the root to the shoot contribute to the overall salt tolerance in glycophytes (Zingarelli et al., 1994; Niu et al., 1995).

CONCLUSIONS

1. Despite the fact that salt treated and control plants had both exhibited similar water content in leaf, stem and root, the fresh mass yielded differed greatly among them, particularly under long-term salinity, suggesting that water plant content in itself does not explain the differences observed in growth.

2. Growth reduction of cashew plants in salinity conditions could be the result of disturbing in general plant metabolism, due probable to specific ion toxicity.

3. Cashew plants exhibited reduced capacity for K^+ tissue accumulations under long-term salinity, presumably because plasma membrane have become more leaky due to Ca^{++} displacement by Na^+ which reduces K^+/Na^+ selectivity.

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