Plant growth, accumulation and solute partitioning of four forest species under salt stress

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Abstract: Under NaCl-salinity Na⁺ and Cl⁻ accumulation of shoot and root vary greatly among glycophyte plants; this is mostly due to genetic diversity and has been utilized to distinguish between plant responses to salinity. The current study aimed to evaluate the accumulation and Na⁺ and Cl⁻ tissue partitioning and its effect on dry mass gain and K⁺ accumulation in one-month-old Prosopis juliflora, Leucaena leucocephala, Piptadenia macrocarpa and Mimosa hostilis seedlings grown for 30 days, in sand watered with nutrient solution loading 100 mol m⁻³ of NaCl, in greenhouse. The Na⁺ and Cl⁻ accumulation, but not partitioning between shoot and root, depended on plant species. All the plants accumulated Cl⁻ over Na⁺ both in shoot and root. The K⁺ content of plants did not significantly vary in shoot but decreased in root due to salinity. The ability of these plants to avoid damaging the metabolism due to salinity may result, in part, from a high shoot-K⁺ to Na⁺ ratio. Leucaena leucocephala had the higher decrease of total dry matter (60%) and lower shoot-K⁺ to Na⁺ ratio (0.40), while Prosopis juliflora had lower decrease of total dry matter (15%) and had K⁺ to Na⁺ ratio of shoot about 3 times more. Evidence is presented supporting a role for increased K⁺ to Na⁺ ratios in adaptation of plants to osmotic and ionic stresses.

Key words: salinity, potassium, sodium, chloride

Crescimento, acumulação e distribuição de solutos em espécies florestais sob estresse salino

Resumo: Em condições de estresse salino a acumulação de Na⁺ e Cl⁻, na parte aérea e nas raízes, difere substancialmente entre plantas glicófitas; este fato deve-se, principalmente, à diversidade genética existente e tem sido utilizado como indicador da resposta das plantas à salinidade. Este estudo objetivou avaliar a acumulação e a distribuição de Na⁺ e Cl⁻, bem como o efeito da salinidade sobre o ganho de massa seca e a distribuição de K⁺, na parte aérea e raízes, em plantas jovens de Prosopis juliflora, Leucaena leucocephala, Piptadenia macrocarpa e Mimosa hostilis, com um mês de idade, cultivadas durante 30 dias em areia e irrigadas com solução nutritiva contendo 100 mol m⁻³ NaCl, em casa de vegetação. A acumulação de Na⁺ e Cl⁻, e não a distribuição desses íons entre parte aérea e raízes, foi dependente da espécie de planta. Em todas as espécies estudadas a acumulação de Cl⁻ na parte aérea e nas raízes foi maior que a de Na⁺. A exceção da parte aérea, todas as plantas apresentaram redução significativa no conteúdo de K⁺ nas raízes em resposta à salinidade. A habilidade dessas plantas em proteger o metabolismo do efeito deletério da salinidade pode resultar, em parte, de uma alta relação K⁺/Na⁺. Desta forma, a Leucaena leucocephala apresentou a maior redução na produção da massa seca total (60%) e a menor relação K⁺/Na⁺ na parte aérea (0,40) enquanto que a Prosopis juliflora apresentou a menor redução na massa seca total (15%) e uma relação K⁺/Na⁺ na parte aérea, aproximadamente três vezes maior. Esses resultados sugerem que maiores relações K⁺/Na⁺ desempenham um importante papel na adaptação da planta aos estresses iônico e osmótico.

Palavras-chave: salinidade, potássio, sódio, cloreto
INTRODUCTION

The effects of salinity on plant growth have extensively been a focus of research because of salt response of plants is a complex phenomenon that involves several physiological and biochemical changes (Hasegawa et al., 2000; Gholam et al., 2002). Ionic imbalance occurs in the cell due to excessive accumulation of Na⁺ and Cl⁻ and reduces the uptake of other mineral nutrients such as K⁺ and NO₃⁻ (Viégas et al., 1999). It has been suggested that Na⁺ and Cl⁻ accumulation in root over shoot could be useful as indicator of salinity tolerance of plants (Silveira et al., 2001). The best manifestation of this is exemplified by those cases in which gain in dry mass were associated with decreased accumulation of Na⁺ and Cl⁻ in shoot of some woody plants in the early seedling phase (Teleinskk & Grunberg, 1994; Souza et al., 2002; Fausto et al., 2002).

The exclusion of Na⁺ from shoot and its preferential accumulation in root has been observed in ‘cumaru’ (Souza et al., 2002), ‘algaroba’ (Fausto et al., 2002), ‘umbu’ (Paulo et al., 2002) and cashew (Viégas et al., 1999). In the later case, Na⁺ exclusion occurs from young to either old leaves or root but only in the earlier stages of salinity stress. According to Viégas et al. (1999), this process is disrupted in cashew plants due to displacement of root-Ca²⁺ by Na⁺, resulting in a change of plasma membrane permeability (Lin et al., 1997). This could induce increase in net uptake of Na⁺, allowing down hill influx of Cl⁻ into cell of plant tissues (Skerrtt & Tyerman, 1992). Because of this Na⁺ and Cl⁻ uptake has been suggested to be a process highly coordinated (Viégas et al., 1999).

The Ca²⁺ displacement by Na⁺ at plasma membrane may constitute a primary response to salinity stress. Under this condition K⁺/Na⁺ selectivity may substantially be altered (Serrano & Rodriguez-Navarro, 2001). It has been suggested that the interaction between K⁺ and Na⁺ might represent a key factor in determining the salinity tolerance of plants (Buschmann et al., 2000). 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 salt tolerance of glycophytes (Rascio et al., 2001). Recent research with mutants of Arabidopsis has shown that the integrity of the K⁺ uptake system plays a role to salt tolerance (Zhu et al., 1998). Higher level of K⁺ in leaf tissue is associated with salt tolerance in cowpea (Silveira et al., 2001). The role of K⁺ is vital for osmoregulation and protein synthesis, maintaining cell turgor and stimulating photosynthesis (Buschmann et al., 2000).

Although increase in soil salinity is one of the major problems confronting agriculture in the semi-arid region of Brazil, research result are scarce and has no or limited information about salinity responses of tree-plants existing in that region. Thus, the purpose of this work was to study the accumulation and Na⁺ and Cl⁻ partitioning and the effect of salinity on dry mass gain and K⁺ accumulation in the early seedling phase of Leucaena leucocephala (Benth.), Prosopis juliflora (Benth.), Mimosa hostilis (Benth.) and Piptadenia macrocarpa (Benth.), common names “leucena”, “algaroba”, “jurema” and “angico”, respectively.

MATERIAL AND METHODS

Plant material and growth conditions

Seeds of Prosopis juliflora, Piptadenia macrocarpa, Mimosa hostilis and Leucaena leucocephala were collected from plants grown under field conditions in semi-arid region of Northeastern of Brazil. The seeds were surface sterilized for 15 min in 5% NaOCl and rinsed for 10 min. with running distilled water then sown in sand irrigated daily with 1.0 mol m⁻³ CaSO₄ (Silveira et al., 2001). After emergence, 15-day-old homogeneous seedlings of each species were transplanted to plastic pots (20 cm in long x 30 cm high) filled with 0.5 cm³ of sand. The pots were kept in well-ventilated greenhouse during the experimental period. Each pot was irrigated daily with 1/4 strength Hoagland & Arnon (1950) nutrient solution. When seedlings were one-month-old, irrigation was carried out daily with half strength nutrient solution loading 100 mol m⁻³ of NaCl during 30 days. Plants irrigated with nutrient solution free of NaCl were taken as control. The pots were irrigated twice a day until leaching started to avoid salt accumulation. The pH of nutrient solution was kept in 5.5±0.2 range with HCl or NaOH. The study was carried out without shelter in semi-arid region of Brazil during September/October months. The air temperature and relative humidity in the greenhouse were in the range of 26 to 33 ºC and 35 to 50%, respectively. The maximal intensity of radiance at plant canopy was of 1,500 µmol of photons m⁻². s⁻¹. After treatment period, the plants were separated into root and shoot. The roots were washed with distilled water, then both plant parts were dried at 90 ºC and properly stored for further analysis of Na⁺, K⁺ and Cl⁻.

The experiment was conducted in a 2 x 4 factorial completely randomized design corresponding to NaCl levels and plant species, respectively, and had six replicates per treatment, each replicate consisting of one plant per pot.

Na⁺, K⁺ and Cl⁻ determination

Dried plant tissue was powdered and subjected to wet digestion with (4:1) HNO₃ : HClO₄ (v/v) according to Chapman & Pratt (1961). The resulting solutions were properly diluted and analyzed for K⁺ and Na⁺ by flame photometer. Cl⁻ was extracted from the dried tissue with water and determined by titration against standard AgNO₃ (Malavolta et al., 1989).

Data analysis

The effects of treatments on plant dry mass accumulation and ion content were performed by ANOVA system. The treatment means were compared by LSD (least significant difference) at the 0.05 confidence level using Tukey test. Standard errors (SE) are reported in the figures. ANOVA results were calculated with the SIGSTAT package.

RESULTS AND DISCUSSION

Seeds of four forest species (Prosopis juliflora, Piptadenia macrocarpa, Mimosa hostilis and Leucaena leucocephala) were grown in 100 mol m⁻³ of NaCl and were evaluated for the early responses to salinity. Salinity provoked
slight yellowing on leaves already from the 10th day of treatment in *Mimosa hostilis* and *Leucaena leucocephala*. The salt symptom was aggravated with increasing exposure time of salinity; the leaves of these two plants exhibited severe necrotic and chlorotic symptoms. In addition, there was significant interaction ($P < 0.05$) between salinity (S) and plant species (E) factors for all studied parameters given below, and this is largely expected due to great genetic diversity of the investigated plants. Particularly, information was sought on the role of these interactions concerning the earlier responses of the plants to salinity.

Dry matter (DM) accumulation of NaCl grown plants is summarized in Figure 1. The DM gain remarkably differed among plants; for instance, NaCl did not significantly ($P > 0.05$) affect total DM of *Prosopis juliflora* and *Piptadenia macrocarpa* contrary to *Mimosa hostilis* and *Leucaena leucocephala* (Figure 1A). Total DM of *Mimosa hostilis* and *Leucaena leucocephala* had a decrease of 42 and 60%, respectively, compared to respective controls. *Prosopis juliflora* and *Leucaena leucocephala* had the more contrasting results in terms of dry mass partitioning. *Prosopis juliflora* had a decrease in DM of 14 and 5% in shoot and root, respectively compared to controls. Instead, salinity accounted for a decrease in DM in shoot and root of *Leucaena leucocephala* by 61 and 52%, respectively (Figure 1B, 1C).

The accumulation and Cl, Na$^+$ and K$^+$ partitioning both in tissues and organs are important physiological processes leading to salt tolerance (Schachtman & Liu, 1999; Serrano & Rodriguez-Navarro, 2001). Among the studied species, *Leucaena leucocephala* was observed to accumulate higher amounts of Na$^+$ and Cl$^-$ in shoot. Na$^+$ accumulated in shoot of *Leucaena leucocephala* reached an amount about 2.6-fold higher than the other species (Figure 2A). This indicates for excessive low-affinity Na$^+$ uptake, which is toxic to
the growth of glycophytic plants (Buschman et al., 2000). In addition, *Leucaena leucocephala* had the highest content of Cl$^-$ in shoot compared to the other species (Figure 2B). This may inhibit some enzymes mostly those involved with NO$_3^-$ to NH$_4^+$ reduction (Viégas & Silveira, 1999). The results from Figures 2A, 2B show still that *Prosopis juliflora* and *Piptadenia macrocarpa* had the lower contents of Na$^+$ and Cl$^-$ in shoot.

Reports have suggested that interaction between internal K$^+$ and Na$^+$ might represent a key factor in determining the salinity tolerance of plants (Rascio et al., 2001; Silveira et al., 2001). In the current study, the shoot-K$^+$ content of plants have not been significantly ($P > 0.05$) changed due to salinity (Figure 2C). In addition, *Prosopis juliflora* had the higher content of K$^+$ in shoot compared to the other ones. On the contrary, increasing in accumulation of salt ions in tissue, particularly Na$^+$, due to salinity, significantly affected the K$^+$ content in root of the plants (Figure 2A, B, C). There are physiological evidences suggesting that Na$^+$ competes with K$^+$ for intracellular influx since both cations share common transporter protein (Hasegawa et al., 2000).

*Prosopis juliflora* had shoot K$^+$ to Na$^+$ ratio of 1.16 while *Leucaena leucocephala* had of 0.40 (Figure 3A). Bottacin et al. (1984), encountered K$^+$ to Na$^+$ ratios in shoot of 1.46 for the tolerant and of 0.64 for the salt susceptible genotypes; these authors concluded that salt tolerance to be characterized by the ability of plants to maintain K$^+$ to Na$^+$ ratios of shoot adjusted to K$^+$ metabolic requirements. Thus, the salt tolerance of plants is due partly to its ability to avoid accumulation of harmful Na$^+$ and/or maintain adequate levels of K$^+$ in shoot (Lacerda et al., 2001). In the most general terms, the highest Na$^+$ shoot to root ratio observed for *Leucaena leucocephala* show the roots of this plant were comparatively less able to prevent harmful Na$^+$ from reaching the leaves (Figure 3B).

In the present study the higher sensitivity to salinity of *Mimosa hostilis* and *Leucaena leucocephala*, in terms of total dry matter gain, could be due partly to the highest contents of Na$^+$ and Cl$^-$ in shoot and root of these plants (Figures 1A, 2A e B). It is interesting to show a preferential accumulation of Cl$^-$ over Na$^+$ both in shoot and root of the four studied species (Figures 2A e B). This is likely due to increased uptake and furthermore Na$^+$ accumulation, possible depolarizing the ÅØ across plasma membrane which, according to Skerrett & Tyerman (1992), allows that CI be taken up passively through an anion channel.

It has been established for plant root a dual uptake system of K$^+$ with either high or low affinity for K$^+$ that adjust with environmental stimulus (Niu et al., 1995; Maathuis et al. 1996). It is possible, as it was seen for NaCl-stressed *Saccharomyces cerevisiae* cells (Serrano & Rodriguez-Navarro, 2001), that in plants with high K$^+$ to Na$^+$ ratios the K$^+$ uptake system changes to a state in which there is an increased affinity for K$^+$ thereby effectively reducing Na$^+$ influx. Thus, a lower accumulation of Na$^+$ in shoot of *Prosopis juliflora* and *Piptadenia macrocarpa* points toward increased K$^+$ selectivity of the K$^+$ uptake system. The contrary is expected for *Mimosa hostilis* and *Leucaena leucocephala*. Indeed, presumably genotypes that are most tolerant to salinity tightly regulate ion uptake across plasma membrane at rates compatible to capacity for vacuolar compartmentalization and growth at sizeable rates (Yeo & Flowers, 1983; Flowers & Hajibagheri, 2001). In addition, transport process at the plasma membrane and tonoplast that regulates ion influx and efflux, particularly those involved in the control of uptake and Na$^+$ vacuolar compartmentalization, are of great significance for salinity metabolic adjustment (Maathuis et al., 1996). Thus, maintenance of ion homeostasis compatible to sustained growth may result from inside negative membrane potential which, according to Binzel et al. (1988), creates a substantial thermodynamic barrier to the Cl$^-$ downhill influx even at relatively high external concentrations of this ion.

In the current study decrease in accumulation of dry matter of shoot of NaCl treated plants was greater than the root (Figure 1B, 1C). This is expected and has been found for a number of glycophytes species (Munns & Termaat, 1986; Chessemann, 1988; Viégas et al., 2001), and it is expected to be due partly to decreasing photosynthetic rates and due partly to increasing the export of carbohydrate from shoot to root (Silveira et al., 2001).

**CONCLUSIONS**

1. In terms of dry matter gain *Prosopis juliflora* and *Piptadenia macrocarpa* responded best to salinity than *Mimosa hostilis* and *Leucaena leucocephala* seedlings.

2. The higher reduction in dry matter of *Mimosa hostilis* and *Leucaena leucocephala* may result from a higher accumulation in shoot of Na$^+$ and Cl$^-$ and from a lower shoot-K$^+$ to Na$^+$ ratio.

3. The pattern of Na$^+$ and Cl$^-$ partitioning between shoot and root was similar for all the plants.

4. In the early seedling phase, *Prosopis juliflora* and *Piptadenia macrocarpa* species seem to have a mechanism to avoid over accumulation of Na$^+$ and Cl$^-$. 

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**Figure 3.** K$^+$ to Na$^+$ ratios of shoot (A) and Na$^+$ shoot to root ratios (B) of *Prosopis juliflora* (Pj), *Piptadenia macrocarpa* (Pm), *Mimosa hostilis* (Mh) and *Leucaena leucocephala* (Ll) grown in 100 mol m$^{-3}$ of NaCl. Means followed by the same letter are not significantly different at $P > 0.05$ (Tukey test). DM, indicates dry mass. Upper bars give ± SD.
ACKNOWLEDGEMENT

To Conselho de Desenvolvimento Científico e Tecnológico (CNPq) for fellowship and financial support given to Ricardo Almeida Viégas and Joaquim Albenísio Gomes Silveira.

LITERATURE CITED


