

Growth and ion accumulation in seedlings of
Handroanthus serratifolius (VAHL.) cultivated in saline solutionCrescimento e acúmulo de íons em plântulas de
Handroanthus serratifolius (VAHL.) cultivadas em solução salinaFabricio José Pereira¹ e Marcelo Polo²**Resumo**

A salinidade é um problema ambiental para diversos biomas e muitas plantas precisam suportar seus efeitos para sobreviverem nessas condições. Entretanto, algumas espécies nativas não são capazes de lidar com tais ambientes e devem ser conhecidas. Este trabalho teve como objetivo avaliar a tolerância à salinidade de *Handroanthus serratifolius*, uma espécie pioneira muito utilizada em reflorestamentos. Plântulas com 15 dias de idade de *Handroanthus serratifolius* foram cultivadas por 45 dias em solução nutritiva e tratamentos salinos com NaCl com potenciais osmóticos de -0,1, -0,2, -0,3, -0,4, -0,8 e -1,5 MPa. O acúmulo de sódio foi quantificado nas plântulas com fotômetro de chama e o cloro por titulação. As variáveis analisadas foram: biomassa seca da raiz, caule e folhas e a área foliar sendo utilizadas para calcular os índices de crescimento sendo: taxa de crescimento relativo, taxa assimilatória líquida, razão raiz:parte aérea. Foi avaliada ainda a densidade estomática na face abaxial das folhas. Os dados foram submetidos à ANOVA com cinco repetições. As plântulas sobreviveram apenas nos tratamentos com -0,1 (92% de sobrevivência), -0,2 (56% de sobrevivência), -0,3 MPa (32% de sobrevivência) e no grupo controle (100% de sobrevivência). A biomassa seca das plântulas foi reduzida em todos os tratamentos salinos em comparação com o grupo controle. A salinidade promoveu decréscimo na taxa de crescimento relativo e na taxa assimilatória líquida. A área foliar e a densidade estomática foram reduzidas em todos os tratamentos salinos. Todos os tratamentos salinos aumentaram a razão raiz:parte aérea. As plântulas acumularam sódio proporcionalmente às soluções salinas mas o cloro acumulou apenas até valores de 0,41 m Kg⁻¹ na solução de -0,1 MPa não diferindo nas soluções com menores potenciais osmóticos. Dessa forma, *Handroanthus serratifolius* não pode sobreviver em condições severas de salinidade e o desenvolvimento da plântula só é possível em potenciais osmóticos de até -0.3 MPa.

Palavras-chave: Cloreto de sódio, Densidade estomática, Estresse osmótico, Taxa de crescimento relativo

Abstract

Salinity is an environmental problem in several biomes and many plants have to overcome its effects in order to survive. However, some native species are not able to cope with such environment and this must be known. This work evaluated the salt tolerance of *Handroanthus serratifolius*, a well known pioneer species in reforestation. Fifteen days old seedlings of *Handroanthus serratifolius* were grown for 45 days in nutrient solution and NaCl treatments of -0.1, -0.2, -0.3, -0.4, -0.8 and -1.5 MPa osmotic potentials. Sodium accumulation was quantified in whole seedlings by flame photometer and chloride by titration. The evaluated variables were: root, stem and leaf dry biomass as well as leaf area, and these were used to calculate the growth parameters: relative growth rate, net assimilatory rate and root:shoot ratio. Stomata density was evaluated on the leaves' abaxial face. Statistical analysis was performed in one-way anova with five replicates. Seedlings survived only at -0.1 (92% of survival), -0.2 (56% of survival) and -0.3MPa (32% of survival) and in the control group (100% of survival). Seedlings dry biomass declined in all saline treatments compared to the control group. Salinity promotes a decrease in relative growth rate and in net assimilatory rate. Leaf area and stomata density were reduced in all saline treatments. All saline treatments increased the root:shoot ratio. Seedlings accumulated sodium proportionally with the saline solutions but chloride accumulate only to 0.41 MPa in the -0.1 MPa solution without differences in the lower osmotic potential solutions. Therefore, *Handroanthus serratifolius* can not survive in severe saline environments, and seedling development is only possible up to an osmotic potential of -0.3 MPa.

Keywords: Sodium chloride, Stomatal density, Osmotic stress, Relative growth rate

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INTRODUCTION

Salinity is one of the major factors in reducing plant growth and productivity. Salinity effects are more visible in arid, semi-arid and irrigated lands (AZEVEDO NETO *et al.*, 2004). Salinization consists of salt accumulation in the soil after water evaporation and salt stress essentially results in a water deficit condition in the plant and takes on the form of a physiological drought (TAWFIK, 2008).

Salinity inhibition of plant growth is the result of osmotic and ionic effects and depends on the plant species (MUNNS, 2002). Salinity can induce an oxidative stress and a reduction in plant growth (KATSUHARA *et al.*, 2005) and can inhibit photosynthetic enzymatic processes (ARAGÃO *et al.*, 2005). Some studies have shown that salt stress inhibits PSII activity (ZURITA *et al.*, 2005) and that the stomata conductance, stomata density and transpiration rate were reduced with the increase of salinity (CABRAL *et al.*, 2004; QIU *et al.*, 2007, SENA *et al.*, 2006).

Researches with Brazilian forest species to define plant resistance to abiotic stress are recent. Water stress studies were conducted with *Tabebuia aurea* (CABRAL *et al.*, 2003), and toxic metals effects on the nutrient uptake were conducted with *Handroanthus impetiginosus* (PAIVA *et al.*, 2004). These two species, closely related to *H. serratifolius*, exhibited tolerance to water stress and toxic metals, and have the same wide distribution such as *H. serratifolius*, indicating a potential tolerance to stress in these plants.

Handroanthus serratifolius (Bignoniaceae) is a tree characteristic of the Brazilian savannah, adapted to drought and poor lands, known mainly as "ipê amarelo da mata" (GROSE; OLMSTEAD, 2007; LORENZI, 1992). Its wood can be used for several purposes and the plant is recommended for landscape use and restoration of degraded areas (LORENZI, 1992; SANTOS *et al.*, 2009). Species of *Handroanthus* are important in reforestation and some management is necessary for the correct establishment of the species (like soil scarification) due to germination and seedling growth (SANTACRUZ *et al.*, 2006; SANTOS *et al.*, 2009). Some *Handroanthus* species are representative in endemic biomes, in reforested areas and in areas subject to agro-forestry systems (MERLOS *et al.*, 2005). *H. serratifolius* has a potential for restoration of areas under salinity. This capacity can be due to its wide distribution and the wide soil conditions that this species colonizes.

The objective of this investigation was to evaluate the effects of salt stress on growth and stomata density of *Handroanthus serratifolius* trees, and to correlate these effects with changes in ionic solute accumulation.

MATERIALS AND METHODS

Seeds of *Handroanthus serratifolius* (Vahl.) collected from trees cultivated in southeastern Brazil, Minas Gerais state, were surface sterilized with 0.02% CaCl₂ solution for 10 min. and rinsed in tap water. Seeds were germinated on moist Whatman filter paper moistened with distilled water in four replicates of 150 seeds. Following 15 days germination in a 30°C growth chamber, uniform seedlings were transferred to pots containing 2 L of nutrient solution in five replicates of 100 seedlings (500 seedlings). The plants were grown under controlled growth chamber (B.O.D.) conditions (light/dark regime of 12/12h, provided by five fluorescent lamps with photosynthetically active radiation (PAR) flux of 89 mmol.m⁻².s⁻¹, at 25°C and a relative humidity of 90%). Nutrient solutions were changed every 3 days; distilled water was used in the preparation of the nutrient solutions (EKER *et al.*, 2006). Ten days after transferring seedlings to nutrient solution they were transferred to plastic pots containing 2 L of half-strength Hoagland's (control treatment) or nutrient solution to which NaCl was added according to Table 1 (salt stress treatment). The osmotic potential was obtained based on the Van't Hoff equation ($\psi_{os} = -nRT$, where "n" is the molar concentration of particles; R is the Gas constant and "T" is the temperature in °K), in five replicates of 50 seedlings.

After 45 days plants from all the treatments were harvested; separated into stem, leaf and root parts for fresh and dry biomass determinations as well as for leaf area measurements for growth analysis. Dry biomass was determined after drying the parts in an oven at 80°C for 72h, while leaf area was measured through the UTHSCSA-imagetool software. Growth analysis was conducted according to following physiological index: relative growth rate (RGR= LnM2-LnM1/T2-T1), leaf area ratio (LAR= Leaf area/whole plant biomass) and net assimilation rate (NAR= RGR*LAR). Stomata density was determined according to Martins *et al.* (2009) in the two first full expanded leaves, on the abaxial leaf surface in five plants by replicate.

Table 1. Sodium chloride molar concentration and osmotic potential of saline solutions used for cultivation of *H. serratifolius* during 45 days.

Tabela 1. Concentração molar de cloreto de sódio e potencial osmótico de soluções salinas utilizadas para cultivo de *H. serratifolius* durante 45 dias.

Treatment	Molar Concentration (M)	Osmotic potential (MPa)
Control	--	0.0048
A	0.019	-0.1000
B	0.038	-0.2000
C	0.057	-0.3000
D	0.078	-0.4000
E	0.160	-0.8000
F	0.320	-1.5000

Sodium analyses were made with a flame photometer after the samples had been burnt to ashes at 700°C in platinum crucibles, immediately allowed to cool, and then had been taken up in 0.16 M HNO₃. Flame photometer readings were made at 586 nm. Chloride was measured by the titration method with mercuric nitrate (SCHALES; SCHALES, 1941).

The results were submitted to statistical analysis by the one-way ANOVA and Tukey's test for the salinity accumulation at a significance level of 5% or the regression analysis for growth parameters in Sisvar statistical software, in five replicates of 50 seedlings for each treatment in a totally randomized statistical design.

RESULTS AND DISCUSSION

The percentage of seedling survival rate was significantly reduced under salinity. Under -0.1 MPa survival was uniformly high, averaging 92 percent. Seedlings of treatment -0.2 MPa averaged 56 percent survival, while those of treatment -0.3 MPa averaged only 32 percent. More intense salinity (-0.4, -0.8 and -1.5 MPa) resulted in death of all individuals.

Salinity had a marked effect on dry matter production per plant (Figure 1 A). Biomass of the *H. serratifolius* seedlings declined sharply with salinity up to -0.1 MPa followed by stabilization on the -0.2 and -0.3 MPa treatments.

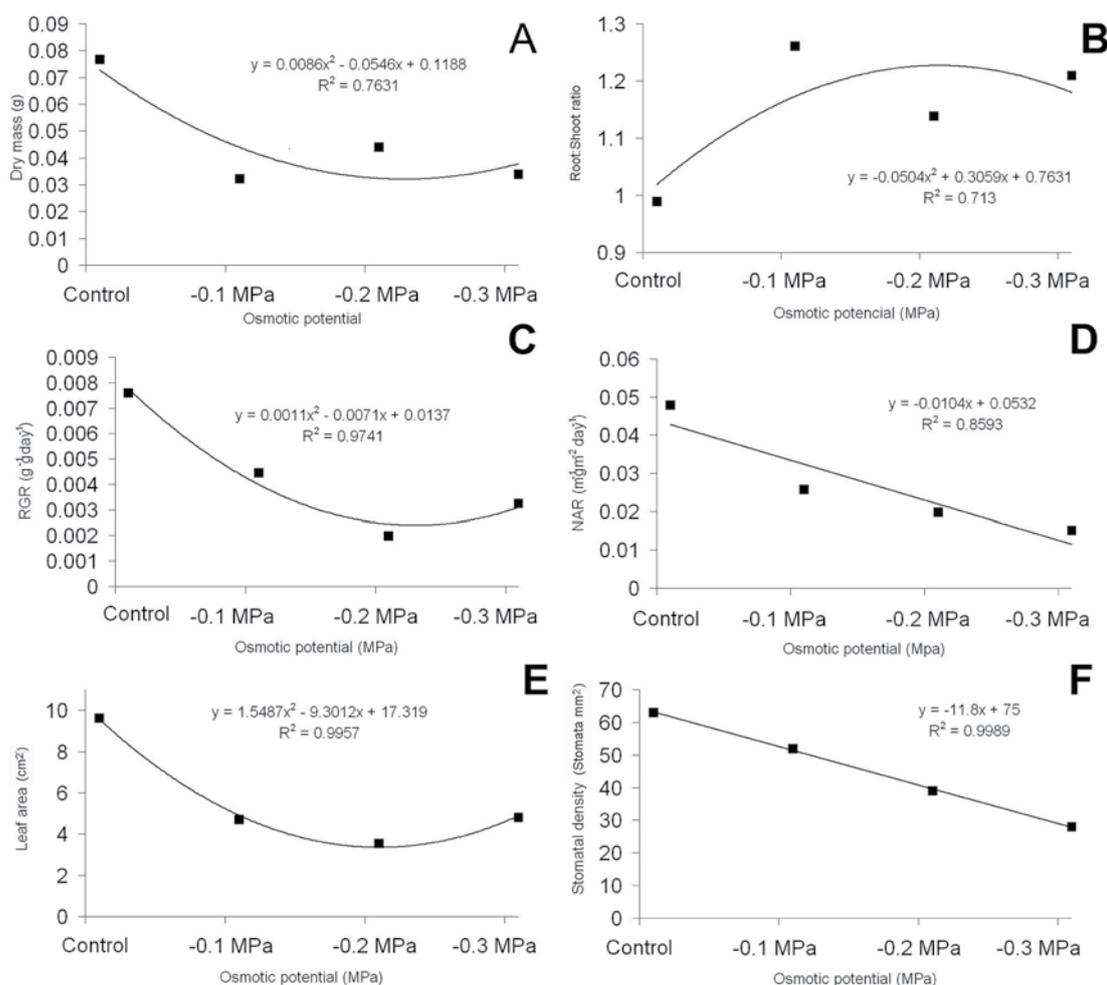


Figure 1. Total dry biomass (A); shoot:root ratio (B); RGR, relative growth rate (C); NAR, net assimilation rate (D); leaf area (E) and stomata density (F) of *Handroanthus serratifolius* seedlings grown under control and salt stress conditions.

Figura 1. Biomassa seca total (A); relação raiz:parte aérea (B); TCR, taxa de crescimento relativo (C); TAL, taxa assimilatória líquida (D); área foliar (E), e densidade estomática (F) de plântulas de *Handroanthus serratifolius* em condições de estresse salino e controle. As barras representam as o desvio padrão da média.

The exposure to increasing salinity resulted in significantly higher mean root:shoot ratios for *H. serratifolius* plants (Figure 1 B). The root:shoot ratio increased above -0.1 MPa, followed by non significant oscillation on the other treatments.

RGR decreased with increase in solution salt concentration (Figure 1 C). An observation of (Figure 1 D) shows that NAR decreased with saline treatments and the maximum NAR was found in the control treatment.

Leaf area decreased gradually with -0.1 MPa, -0.2 MPa and -0.3 MPa, and there was a relative increase from the -0.2 MPa to -0.3 MPa treatments (Figure 1 E). Stomata density under the epidermis in leaves of plants was also significantly reduced with a salinity increase (Figure 1 F).

Sodium and chloride content in the control group was low. Increasing salinity levels led to a larger sodium level in the seedlings (Table 2). Mean sodium content increased 7.2 times in the -0.1 MPa treatment (Table 2); in the -0.2 MPa sodium content was 53.26% higher than in -0.1 MPa and in the -0.3 MPa treatment sodium content was on the average 11,11% higher than in the -0.2 MPa treatment (Table 2). Chloride content increased in the -0.1MPa treatment, and then stabilized (Table 2).

Table 2. Accumulation of Na⁺ and Cl⁻ (mol.kg⁻¹) in the seedlings of the *Handroanthus serratifolius*, growing in nutrient solutions containing NaCl with osmotic potentials of -0.1, -0.2, -0.3 MPa and control group without NaCl.

Tabela 2. Acúmulo de Na⁺ e Cl⁻ (mol.kg⁻¹) em plântulas de *Handroanthus serratifolius* crescendo em soluções nutritivas contendo NaCl com potenciais osmóticos de -0,1, -0,2, -0.3 MPa e grupo controle sem NaCl.

Treatments	Na ⁺	Cl ⁻
Control	0.05 d	0.20 b
-0.1 MPa	0.41 c	0.41 a
-0.2 MPa	0.63 b	0.40 a
-0.3 MPa	0.70 a	0.40 a

Means followed by the same letter in columns do not differ significantly (Tukey's test, p<0.05).

This attempt to elucidate the response *H. serratifolius* species to salinity led to a considerable decline in survival and growth parameters with increasing salinity levels. This may be due to poor tolerance in root hardiness of this species to such an adverse environment.

The decrease in total dry mass of the seedlings from control treatment revealed only a mild salinity tolerance of *H. serratifolius*. Costa *et al.* (2003), while studying the performance of different *Vigna unguiculata* cultivars in salt-

affected soils found a decrease in dry biomass. Our results were in conformity with the findings of Melloni *et al.* (2000), in *Myracrodruon urundeuwa* plants. *H. serratifolius* responses to salinity were similar to the responses in other plant species.

The allocation of dry mass to plant parts indicated a pattern which was consistent with the findings of Costa *et al.* (2003), Ewe and Sternberg (2005), and Tewari *et al.* (2006) where root:shoot ratio had an inverse relation to RGR and NAR. In those studies, all species increased root:shoot ratio under salinity and this may be due to the capacity of longer roots being able to reach areas of lower salinity and more available water. *H. serratifolius* may partially cope with mild salinity by increasing its root:shoot ratio in an effort to get more water.

The reduction on the NAR, biomass, RGR and leaf area in the present work supports the correlation between the photosynthetic capacity and leaf area. This is in agreement with the hypothesis of Reich *et al.* (1999) that no species can improve its photosynthetic capacity without increasing leaf area due to biophysical limitations. Thus salinity causes limitations on *H. serratifolius* photosynthesis and this is associated with a reduction on growth parameters as well as with total biomass production. Reduction of photosynthesis can be due to stomata limitations (by the reduction in its density, as verified in this work, and by reduction in the stomata conductance) and by non stomata limitations (by enzymatic inhibition due to toxicity of sodium and chloride). In this work both of these effects may have occurred but only stomata density reduction was verified.

Reductions on stomata density induce differences on photosynthesis and transpiration due to the increase in salinity. The decrease in photosynthesis may result from the restriction of CO₂ uptake by stomata, reducing stomata conductance (QIU *et al.*, 2007). *H. serratifolius* photosynthesis may be partially reduced by a lowered CO₂ uptake and this lead to reductions on growth parameters under mild salinity.

Sodium content strongly increased in all seedlings with increasing NaCl concentration in the nutrient solution. This is consistent with the findings of Costa *et al.* (2003) and Lacerda *et al.* (2001). A common behavior observed in non salinity-tolerant plants is that higher levels of sodium in cells promote enzymatic inhibitions. Chlorine is an essential micronutrient for higher

plants, and differences between cultivars to withstand Cl⁻ toxicity are frequently related to the ability to restrict Cl⁻ transport to the shoot (WHITE; BROADLEY, 2001). This restriction of transport to the shoot may be related to an increase in apoplastic barriers such as endodermis in roots, which can be increased under stress (PEREIRA *et al.*, 2008; SOUZA *et al.*, 2009). It is possible that *H. serratifolius* capacity to withstand mild salinity may be due to the restriction on Cl⁻ accumulation on plants.

CONCLUSION

Salinity reduced seedling growth in *Handroanthus serratifolius* and this is due to a decreased leaf area for photosynthesis, NAR, biomass and RGR and toxic effects of accumulation of sodium and chloride. This species can't survive under osmotic potentials lower than -0.3 MPa.

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