RESPONSE TO SALT STRESS IN GROWTH, WATER RELATIONS, AND ION CONTENT OF Jatropha curcas AND J. cinerea SEEDLINGS

Masako Hishida, Felipe Ascencio, Hideyasu Fujiyama, Andrés Orduño-Cruz, Tsuneyoshi Endo and Juan A. Larrinaga-Mayoral

SUMMARY

Saline stress on Jatropha curcas, a plant native to humid tropical areas, was assessed to determine its potential for cultivating it as a biodiesel crop in arid and saline areas and compared with Jatropha cinerea, a wild species of saline dry areas. J. curcas and J. cinerea were subjected to four NaCl concentrations (0, 50, 100 and 200mM) for 28 days, and the effects on growth, ion relations, water potential and stomatal conductance were measured. Both species had the capacity to regulate and maintain water uptake. Stomatal conductance decreased in approximately the same amount in both species. Chlorophyll content decreased only in J. curcas. Biomass production was strongly affected in both species, probably as a consequence of reduced stomatal conductance and ion toxicity. Biomass production and ion relations responded similarly at 50mM, but salinity inhibited J. curcas more than J. cinerea at 100mM from larger Na⁺ uptake and nutritional disorder. Young J. curcas plants have the capacity to grow in dry areas when soils are moderately saline.

RESPUESTA AL ESTRÉS SALINO EN CRECIMIENTO, RELACIONES HÍDRICAS Y CONTENIDO IÓNICO DE PLÁNTULAS DE Jatropha curcas Y Jatropha cinerea

Masako Hishida, Felipe Ascencio, Hideyasu Fujiyama, Andrés Orduño-Cruz, Tsuneyoshi Endo y Juan A. Larrinaga-Mayoral

RESUMEN

Se estudió el efecto del estrés salino en Jatropha curcas, una planta nativa de áreas tropicales húmedas, para determinar su potencial como cultivo en zonas áridas salinas para producción de biodiesel, y compararlo con Jatropha cinerea, una especie silvestre de zonas áridas salinas. Tanto J. curcas y J. cinerea fueron expuestas a cuatro concentraciones de NaCl (0, 50, 100 y 200mM) durante 28 días, siendo medidos los efectos en el crecimiento, relaciones iónicas, potencial hídrico y conductancia estomática. Ambas especies mostraron capacidad de regular y mantener su consumo de agua. La conductancia estomática decreció en una magnitud aproximadamente igual en las dos especies. El contenido de clorofila disminuyó solamente en J. curcas. La producción de biomasa fue afectada fuertemente en ambas especies, probablemente como consecuencia de la conductancia estomática reducida y toxicidad iónica. La producción de biomasa y las relaciones iónicas respondieron de forma similar ante 50mM, pero la salinidad inhibió más a J. curcas que a J. cinerea a 100mM debido a una mayor captación de Na⁺ y desorden nutricional. Plantas juveniles de J. curcas son capaces de crecer en zonas áridas cuando los suelos son moderadamente salinos.

Introduction

Soil salinity inhibits germination, plant growth and productivity (Sairam et al., 2002). Over 8×10⁸ha, about 6% of the world’s land area, is affected by salinity (Munns, 2005). At least 20% of the irrigated land is declining in productivity, a fact related to salinity (Munns and Tester, 2008). Salinization is increasing in semi-arid and arid regions with increasing drought, high evapotranspiration, higher temperatures and inadequate agriculture management (Meloni et al., 2003). Demand for food, fiber, and energy increases the use of saline soils. One strategy to extend the range of cultivated land is to use naturally salt-tolerant species (Maggio et al., 2000).

Saline solutions impose osmotic and ionic stress in plants and reduce their ability to take up water (Ghoulam et al., 2002; Munns, 2002). This water deficit quickly causes a reduction in growth rate, due to a decrease of cell expansion and cell division (Munns, 2002). During periods of water deficit, stomatal conductance decreases in order to maintain osmotic potential and prevents excessive salt accumulation.

KEYWORDS / Cation Balance / Jatropha curcas / Na⁺ Uptake / Salinity / Water Relations /

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RESPONSA AO ESTRESSE SALINO EM CRESCIMENTO, RELAÇÕES HÍDRICAS E CONTEÚDO IÔNICO DE PLÂNTULAS DE Jatropha curcas E Jatropha cinerea
Masako Hishida, Felipe Ascencio, Hideyasu Fujiyama, Andres Orduño-Cruz, Tsuneyoshi Endo e Juan A. Larrinaga-Mayoral

RESUMO

Estudou-se o efeito do estresse salino em Jatropha curcas, uma planta nativa de áreas tropicais úmidas, para determinar seu potencial como cultivo em zonas áridas salinas para produção de biodiesel, e compará-la com Jatropha cinerea, uma espécie silvestre de zonas áridas salinas. Tanto J. curcas e J. cinerea foram expostas a quatro concentrações de NaCl (0, 50, 100 e 200mM) durante 28 dias, sendo medidos os efeitos no crescimento, relações iônicas, potencial hídrico e condutância estomática. Ambas as espécies mostraram capacidade para regular e manter seu consumo de água. A condutância estomática decresceu em magnitude aproximadamente igual nas duas espécies. O conteúdo de clorofila diminuiu somente em J. curcas. A produção de biomassa foi afetada fortemente em ambas as espécies, provavelmente como consequência da condutância estomática reduzida e toxicidade iônica. A produção de biomassa e as relações iônicas responderam de forma similar ante 50mM, mas a salinidade inibiu mais J. curcas que J. cinerea a 100mM devido a uma maior captação de Na+ e desordem nutricional. Plantas juvenis de J. curcas são capazes de crescer em zonas áridas quando os solos são moderadamente salinos.

acumulação (Sultana et al., 1999). Em solos afetados por Na+ e Cl− acumulação em feixes vegetais causa necrose de folha e pode induzir defolição (Tester e Davenport, 2003). Outrossim, os níveis apoplásmicos de Na+ induzem um déficit de ions essenciais (K+, Ca2+ e Mg2+) por competição com Na+ (Niu et al., 1995; Song e Fujiyama, 1996; Blumwald, 2000). Portanto, o balanço de Na+ e ions essenciais na planta pode ser usado como indicador de homeostase nutricional em condições de alta salinidade (Kudo et al., 2010). Em solos com alto teor de sal, plantas protegem-se controlando Na+ e Cl− pela alteração de seu metabolismo e por absorção nos estomas (Hasegawa et al., 2000). Na salinidade, plantas também acumulam Na+ em vacúolos, ajustando o potencial osmótico e regulando a distribuição de água do sistema radicular (Kudo et al., 2010).

Jatropha curcas (‘Barbados nut’, ‘physic nut’) é uma planta tropical e subtropical de crescimento decidual que cresce de 3-5m (Maes et al., 2009) em México e América Central (Achten et al., 2008), onde a precipitação anual é de 500-1000mm (Heller, 1996). J. curcas recebeu muita atenção como uma fonte de energia renovável devido à alta produção de óleo (27-40%) (Fairless, 2007), e é usado na produção de biodiesel e na produção de óleo de jatobá. Jatropha curcas é uma das espécies mais adaptadas a climas sub-tropicais e desérticos, e é cultivada em várias regiões do mundo, incluindo áreas de clima seco. A produção de biodiesel a partir de óleo de Jatropha curcas é uma alternativa para a produção de energia renovável e sustentável.

MATERIALS AND METHODS

PLANT MATERIAL AND GROWTH CONDITIONS

Seeds of Jatropha curcas from Papanal, Veracruz, Mexico, and of Jatropha cinerea from fields near La Paz, Baja California Sur, Mexico, were sterilized with 0.5% sodium hypochlorite for 10min and rinsed three times with distilled water. The seeds were then wrapped with wet paper towels at 25°C for 4 days in the dark to germinate. Uniform first-leaf stage seedlings (10 days after germination) were placed in 4l pots containing 3l of half-strength Hoagland solution for hydroponic cultivation. Salinity treatments were started 14 days after transplanting to the pots. The half-strength Hoagland solution was supplemented with 50, 100, and 200mmol-l−1 NaCl. The salt was added to the solution in one step. The control (0mM NaCl) plants received only half-strength Hoagland solution. Water potential of the saline solution was -0.12 at 0mM NaCl, -0.23 at 50mM, -0.68 at 100mM and -1.57 at 200mM. The solutions were continuously aerated and the pH was adjusted to 5.0 using dilute 1M H2SO4 and 1M NaOH. Water loss of solution by evapotranspiration was compensated by adding water and the solutions were replaced every week. Electric conductivity of the solution was monitored using a portable compact conductivity meter (B-175, Horiba, Kyoto, Japan). Three replicate pots containing four seedlings each were used for each of the four treatments. One of four seedlings in each pot was harvested at the start and 4 days of treatment to monitor plant growth. The other two plants in each pot were cultivated under salt treatment in a naturally illuminated greenhouse for 28 days. Average temperature during cultivation was 22.5°C.

GROWTH ANALYSIS

One plant in each pot was harvested at 28 days to evaluate the salinity effect on biomass production. Harvested plants were washed with distilled water to remove dust and other residues. The plants were separated into leaves, stems and roots, and their fresh weights (FW) measured. Leaf area was measured with a portable leaf area meter (LI-3000A, LI-COR Biosciences, Lincoln, NE, USA). All parts were dried for 48h at 70°C in an oven to obtain dry weight (DW). Total water content (WC) was calculated as

\[
WC(%) = \frac{FW - DW}{FW} \times 100
\]

where WC: water content,
Values are mean ±SD obtained in three plants. The same letters in each column for each species are not significantly different according to the Tukey test after ANOVA (P<0.05).

<table>
<thead>
<tr>
<th>Table I</th>
<th>EFFECT OF NaCl ON DRY WEIGHT (DW), RATIO OF SHOOT TO ROOT (S/R), LEAF AREA, AND LEAF NUMBER IN PLANTS OF J. CURCAS AND J. CINEREA GROWN AT 0, 50, 100 AND 200mM NaCl FOR 28 DAYS</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl (mM)</td>
<td>Root DW (g)</td>
</tr>
<tr>
<td>Jatropha curcas</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.79 ±0.08 a</td>
</tr>
<tr>
<td>50</td>
<td>0.41 ±0.08 b</td>
</tr>
<tr>
<td>100</td>
<td>0.18 ±0.03 c</td>
</tr>
<tr>
<td>200</td>
<td>0.11 ±0.03 c</td>
</tr>
<tr>
<td>Jatropha cinerea</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.48 ±0.19 a</td>
</tr>
<tr>
<td>50</td>
<td>0.31 ±0.10 a,b</td>
</tr>
<tr>
<td>100</td>
<td>0.23 ±0.13 a,b</td>
</tr>
<tr>
<td>200</td>
<td>0.06 ±0.01 b</td>
</tr>
</tbody>
</table>

Values are mean ±SD obtained in three plants. The same letters in each column for each species are not significantly different according to the Tukey test after ANOVA (P<0.05). †: not significant.

**Figure 1.** Effect of NaCl on dry weight (DW) of J. curcas (a) and J. cinerea (b) grown at 0, 50, 100, and 200mM NaCl for 28 days.

**Table 1.** Effect of NaCl on dry weight (DW), ratio of shoot to root (S/R), leaf area, and leaf number in plants of J. curcas and J. cinerea grown at 0, 50, 100, and 200mM NaCl for 28 days.

**Stomatal conductance, transpiration, water potential, and chlorophyll content**

Diffusive stomatal resistance and transpiration of the third leaf from the shoot apex were measured with a porometer (LI-1600, LI-COR Biosciences, Lincoln, NE, USA) in a naturally illuminated greenhouse at 14 days of treatment. Stomatal conductance (gₛ) was calculated as the reciprocal of diffusive stomatal resistance. Mean leaf temperature was 20.8 ±1.3°C and mean photosynthetic photon flux density under greenhouse (PPFD) was 388 ±72μmol·m⁻²·s⁻¹. Measurements were taken between 09:00 and 10:00.

The third fully expanded leaves were collected and immediately placed in zippered plastic bags to measure leaf water potential at day 14 at 10:00. Leaf water potential was determined with a thermocouple psychrometer (WP4-T, Decagon Devices, Pullman, WA, USA). Leaf chlorophyll content index was determined using a chlorophyll meter (SPAD-502, Konica-Minolta, Tokyo, Japan) on the third leaf from the apex once a week for 4 weeks.

**Mineral analyses**

Mineral analysis was performed on oven-dried shoots and roots. Concentrations of Na⁺, K⁺, Ca²⁺, and Mg²⁺ were determined by atomic absorption spectrophotometry.

Gas constant, T: temperature in °K, and V: volume in liters (Song et al. 2009).

**Statistics**

Data were analyzed by one-way ANOVA, and the Tukey post hoc test was used for comparison between the treatments. Statistical significance was set at P<0.05. Computation used Statistica 6.0 (StatSoft, Tulsa, OK, USA).

**Results**

**Salinity effect on biomass production**

Total biomass production in J. curcas and J. cinerea decreased progressively with increasing salt concentrations (Figure 1). The dry weight (DW) of roots, stems and leaves also decreased with increasing salt concentrations, however, growth reduction of leaf was larger than that of stem and root in both species (Table I). The effect of NaCl on shoots tended to be greater than on roots, as shown by the ratio of shoot to root (S/R) of both species (Table I). Comparing the salinity effect, both species had decreased DW similarly at 50mM, but differences in DW between the species occurred at 100mM. In J. curcas it was 16% of the control while it was 32% in J. cinerea. At 200mM, DW of both species decreased to <10% of the control.

Chlorosis and defoliation occurred in the lower leaves at concentrations over 100mM by day 14. Leaf area and number of leaves per plant decreased with increasing concentrations (Table I). Leaf area was affected more than leaf number. Leaf number kept above 50% up to 100mM NaCl in both species, although leaf area was <50% at 50mM in both species.

Stomatal conductance decreased with increasing NaCl concentrations in both species (Figure 2a). At 50mM NaCl, stomatal conductance declined to <50% of the control in both species. At 100mM, stomatal conductance decreased to <25%. Transpiration also decreased with increasing concentrations of NaCl in both species (Figure 2b).

In both species, leaf water potential declined with salt treatment (Figure 2c). In J. curcas, it greatly decreased at 200mM, and in J. cinerea at 100mM.

Total water content significantly increased with increasing salt concentrations (Figure 2d) at 100mM in J. curcas and at 200mM in J. cinerea. Total water content in J. curcas was higher than in J. cinerea at every NaCl concentration tested.
**Salinity effect on chlorophyll content**

The chlorophyll content index declined significantly with salt treatment only in *J. curcas* (Figure 3). Compared to the control, chlorophyll content in *J. curcas* decreased significantly at 50mM NaCl, whereas in *J. cinerea* it did not change significantly with salinity. A strong correlation between dry weight and chlorophyll was observed only in *J. curcas* (P<0.001; Table II). Such strong correlation between chlorophyll content and shoot Na⁺ concentration was also observed only in *J. curcas* (P<0.001).

**Salinity effect on mineral composition**

Under saline treatments, Na⁺ and Cl⁻ concentration in shoots and roots increased with increasing salt concentrations in the substrate solution (Table III). The tendency of Na⁺ concentration under salt treatments was shoot > root in both species. There were rarely differences of Na⁺ concentration in shoots and roots between species at 50mM for DW; however, shoot Na⁺ concentration in *J. curcas* increased markedly at 100mM. The Na⁺ concentration of roots did not change drastically in either species at 100mM compared to the 50mM treatment. Thus, the shoot to root ratio of Na⁺ (Na⁺ S/R) in *J. curcas* doubled at 100 mM from 1.6 at 50 mM, whereas it remained being 1.2 at 50mM and 100mM in *J. cinerea* (Table III). A strong correlation between Na⁺ shoot and root uptake and dry weight was observed in both species (P<0.001; Table II).

Cation imbalance [(Na⁺ / (K⁺ + Ca²⁺ + Mg²⁺))] increased with increasing NaCl concentrations (Figure 4). It was greater in the roots than in the shoots up to 100mM NaCl. Root cation imbalance in *J. curcas* was higher than in *J. cinerea* at every NaCl concentration. As for shoot cation imbalance, *J. curcas* was higher than *J. cinerea* at low salinity levels, but both were similar at higher salinity levels.

**Salinity effect on inorganic ions Ψs**

The calculated osmotic potentials for each inorganic ion are shown in Table IV. Under

### Table II

**Correlation Coefficients of the Measured Parameters in J. curcas and J. cinerea**

<table>
<thead>
<tr>
<th></th>
<th>Dry weight</th>
<th>Na⁺ shoot uptake</th>
<th>Na⁺ root uptake</th>
<th>Stomatal conductance</th>
<th>Water potential</th>
<th>Total water content</th>
<th>Chlorophyll (SPAD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>J curcas</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry weight</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Na⁺ shoot uptake</td>
<td>–0.96**</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Na⁺ root uptake</td>
<td>–0.95**</td>
<td>0.92***</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Stomatal conductance</td>
<td>0.94***</td>
<td>–0.89***</td>
<td>–0.86***</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Water potential</td>
<td>0.50</td>
<td>–0.45</td>
<td>–0.69*</td>
<td>0.39</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Total water content</td>
<td>–0.84***</td>
<td>0.88***</td>
<td>0.79**</td>
<td>–0.74**</td>
<td>–0.28</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Chlorophyll (SPAD)</td>
<td>0.88***</td>
<td>–0.84***</td>
<td>–0.85***</td>
<td>0.76**</td>
<td>0.63*</td>
<td>–0.81***</td>
<td>–</td>
</tr>
<tr>
<td><strong>J cinerea</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Dry weight</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Na⁺ shoot uptake</td>
<td>–0.84**</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Na⁺ root uptake</td>
<td>–0.88***</td>
<td>0.77**</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Stomatal conductance</td>
<td>0.88***</td>
<td>–0.92***</td>
<td>–0.83**</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Water potential</td>
<td>0.83**</td>
<td>–0.61*</td>
<td>–0.75**</td>
<td>0.62*</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Total water content</td>
<td>–0.64*</td>
<td>0.66*</td>
<td>0.67*</td>
<td>–0.73*</td>
<td>0.35</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Chlorophyll (SPAD)</td>
<td>0.42</td>
<td>–0.49</td>
<td>–0.52</td>
<td>0.58</td>
<td>0.21</td>
<td>–0.51</td>
<td>–</td>
</tr>
</tbody>
</table>

*, **, *** denote significance at P= 0.05, 0.01, and 0.001, respectively.
non-salinity condition, the Ψs of K+ were the lowest in shoot and root in both species; however, the Ψs of K+ increased with salinity. Na+ became the most influential ion in root and shoot of both species under salinity. The Ψs of Na+ of J. cinerea declined more in shoot compared with J. cinerea by salinity treatment. In both species, Ψs of Na+ were similar in root, and Ψs of Ca2+ and Mg2+ also increased with salinity in root and shoot.

Discussion

The effect of salinity on growth, water potential and ion relations in seedlings of Jatropha curcas and Jatropha cinerea, which differ largely in their native climate, were measured to study the potential of J. curcas to be cultivated in dry saline lands. Salinity effect on growth was greater in the humid tropical species of J. curcas than in the dry subtropical species of J. cinerea, and the effect became larger with increasing NaCl concentration.

Salinity is known to inhibit photosynthesis in many plant species (Brugnoli and Björkman, 1992; Silva et al., 2010; Suárez 2011). Photosynthesis partly depends on stomatal conductance and chlorophyll content of leaves. As a short-term response to saline soils, plants regulate transpiration flux through reduced stomatal conductance to decrease salinity stress (Munns and Tester, 2008). However, long-term reduction of stomatal conductance induces reduction of photosynthesis because of decreased CO2 availability. Salinity strongly affected stomatal conductance (Figure 2a), and reduction of stomatal conductance was strongly related to biomass production (P<0.001) in both species (Table II), a reduction that could lead to biomass reduction in Jatropha.

Salinity may reduce chlorophyll content by accelerating its degradation (Khan and Abdullah, 2003), which would explain the lower chlorophyll content in J. curcas, because the leaves accumulate large amounts of Na+ and Cl-. Moreover, one explanation of the performance of J. cinerea is that it has a better system to protect chlorophyll than J. curcas, such as vacuolar isolation of Na+. Plants regulate net Na+ flux across the plasma membrane and use vacuolar compartmentalization of internal cations to mediate intracellular Na+ homeostasis (Rus, 2001). J. cinerea could minimize salinity effect on photosynthesis by maintaining chlorophyll content to sustain biomass productivity.

Under salt stress in both species, plants tended to maintain and/or increase water content by decreasing

![Figure 3. Effect of NaCl on chlorophyll content (SPAD value) in plant of J. curcas (○) and J. cinerea (■) grown at 0, 50, 100, and 200mM NaCl for 28 days. The same letters indicate no significant difference (Tukey test after ANOVA, P<0.05). Values are mean of three plants ±SE.](image)

Table III: Effect of NaCl on mineral composition (Na+, Cl−, K+, Ca2+, and Mg2+) in shoot and root and ratio of shoot to root of Na+ (Na+ S/R) in plants of J. curcas and J. cinerea grown at 0, 50, 100, and 200mM NaCl for 28 days

<table>
<thead>
<tr>
<th>NaCl (mM)</th>
<th>Na+ (mmol·g−1) Shoot</th>
<th>Na+ (mmol·g−1) Root</th>
<th>Cl− (mmol·g−1) Shoot</th>
<th>Cl− (mmol·g−1) Root</th>
<th>K+ (mmol·g−1) Shoot</th>
<th>K+ (mmol·g−1) Root</th>
<th>Ca2+ (mmol·g−1) Shoot</th>
<th>Ca2+ (mmol·g−1) Root</th>
<th>Mg2+ (mmol·g−1) Shoot</th>
<th>Mg2+ (mmol·g−1) Root</th>
<th>Na+ S/R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jatropha curcas</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.19±0.01 c</td>
<td>0.15±0.02 d</td>
<td>0.06±0.02 b</td>
<td>0.12±0.04 a</td>
<td>0.94±0.09 a</td>
<td>0.78±0.07 a</td>
<td>0.32±0.06 a</td>
<td>0.23±0.05 a</td>
<td>0.21±0.01 †</td>
<td>0.34±0.11 a</td>
<td>1.3±0.2 b</td>
</tr>
<tr>
<td>50</td>
<td>1.21±0.24 b</td>
<td>0.78±0.05 c</td>
<td>0.27±0.05 b</td>
<td>0.30±0.03 a</td>
<td>0.68±0.11 b</td>
<td>0.58±0.05 a</td>
<td>0.22±0.01 b</td>
<td>0.21±0.04 a</td>
<td>1.14±0.01 †</td>
<td>0.29±0.02 b</td>
<td>1.6±0.2 a</td>
</tr>
<tr>
<td>100</td>
<td>2.43±0.07 a</td>
<td>1.19±0.01 b</td>
<td>0.82±0.20 a</td>
<td>nd</td>
<td>0.63±0.03 b</td>
<td>0.51±0.04 b</td>
<td>0.23±0.01 b</td>
<td>0.18±0.04 a</td>
<td>1.16±0.01 †</td>
<td>0.31±0.02 a</td>
<td>2.0±0.1 a</td>
</tr>
<tr>
<td>200</td>
<td>2.29±0.24 a</td>
<td>1.54±0.41 a</td>
<td>nd</td>
<td>nd</td>
<td>0.67±0.04 b</td>
<td>0.37±0.07 b</td>
<td>0.22±0.01 b</td>
<td>0.10±0.04 b</td>
<td>0.18±0.02 †</td>
<td>0.17±0.03 b</td>
<td>1.5±0.4 b</td>
</tr>
</tbody>
</table>

| Jatropha cinerea |
| 0         | 0.14±0.01 b         | 0.30±0.16 c        | 0.10±0.01 c         | 0.13±0.04 b        | 0.80±0.12 a         | 0.63±0.12 a         | 0.19±0.03 a         | 0.32±0.06 a         | 0.13±0.01 †       | 0.27±0.11 a         | 0.5±0.2 b   |
| 50        | 1.07±0.06 a         | 0.86±0.15 b        | 0.38±0.06 b         | 0.34±0.03 a        | 0.34±0.09 b         | 0.47±0.07 b         | 0.13±0.01 b         | 0.19±0.05 b         | 0.08±0.03 †       | 0.20±0.04 a         | 1.2±0.2 a   |
| 100       | 1.34±0.16 a         | 1.16±0.03 ab       | 0.64±0.09 a         | 0.40±0.09 a        | 0.36±0.07 b         | 0.31±0.02 bc        | 0.15±0.04 a         | 0.15±0.02 a         | 0.09±0.02 †       | 0.20±0.02 a         | 1.2±0.2 a   |
| 200       | 1.28±0.28 a         | 1.58±0.18 a        | nd                  | nd                  | 0.48±0.10 b         | 0.24±0.03 c         | 0.11±0.04 b         | 0.13±0.01 b         | 0.10±0.01 †       | 0.09±0.01 b         | 0.8±0.3 a   |

Values are mean ±SD obtained in three plants. The same letters in each column for each species are not significantly different according to the Tukey test after ANOVA (P<0.05).

n.d.: no data (sufficient material for chloride analysis could not be obtained by reduction of biomass production with salt treatment), †: not significant.
transpiration rate (Figure 2b) and minimizing leaf size (Figure 2d). Under saline stress, plants control their transpiration flux through a better control of leaf anatomy modifications (Abbruzzese et al., 2009). It is known that water content increases to alleviate hyper-ionic stress in succulent plants (Maggio et al., 2000; Khan et al., 2005). Kumar et al. (2008) also reported callus water content of *Jatropha* plant tended to increase under salinity.

Leaf water potential decreased to allow water uptake from saline solution through increase of water potential gradient (Figure 2c). K⁺ represents the main cation in plant cells, and it is an important factor in the cell osmotic potential (Reggiani et al., 1995; Essa, 2002). K⁺ concentration is the highest among cations in the two *Jatropha* species; however, under salinity *Na⁺* was replaced as the major cation composition and the most important component of the cell osmotic potential in plant. Accumulation of Na⁺ contributed to a decrease of leaf water potential, less than that of nutrient solution. Na⁺ may help to maintain turgor but it was unable to substitute Ca²⁺ and K⁺ specific functions such as cellular expansion and enzyme activation for adequate growth (Song and Fujiyama, 1996; Nieves-Cordon et al., 2012; Oueslati et al., 2010).

Biomass production was strongly related with shoot and root Na⁺ uptake in both species (Table II). At 100mM NaCl, Na⁺ content was 2.43 and19 mmol g⁻¹ in shoot and root, respectively, of *J. curcas*. In *J. cinerea*, the corresponding concentrations were 1.34 and 1.16 mmol g⁻¹. Munns (2002) stated that salt-sensitive plants are distinguished by their inability to prevent salt from reaching toxic levels in leaves. Tester and Davenport (2003) mentioned that growth reduction of shoots is affected more than in roots since Na⁺ usually accumulates more in the former. *J. cinerea* appears to be a more tolerant species to salt stress, with a better ability to prevent further accumulation of Na⁺ (Table III).

It is well known that absorption of K⁺ and Ca²⁺ is inhibited when the Na⁺ level is high because the corresponding pathways work as a Na⁺ transporter (Niu et al., 1995). As mentioned above, K⁺ and Ca²⁺ absorption is important to continue plant growth. From the result of a larger cation imbalance in *J. curcas* (Figure 2), salinity stress provoked its serious nutrient disorder due to Na⁺ antagonism. Díaz-López et al. (2012) also suggest that growth reduction in *J. curcas* is related to a nutritional disorder.

In conclusion, the seedling stage of *J. curcas* in a 28-days salinity treatment demonstrated similar potential for growth as those of *J. cinerea* under conditions up to 50mM NaCl, but it is poorly adapted for higher salt accumulation and presents a nutrition disorder at 100mM. The present study suggests that the cultivation potential of *J. curcas* as a cash crop in dry and moderate saline subtropical area is high, although it is still necessary to study the effect on seed production and its oil content before establishing *J. curcas* cultivation in arid and semi-arid areas.

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