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

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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 compararla con* Jatropha cinérea*, 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. cinérea *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 8×10^8ha, 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

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

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RESPOSTA AO ESTRESSE SALINO EM CRESCIMENTO, RELAÇÕES HÍDRICAS E CONTEÚDO IÔNICO DE PLÂNTULAS DE *Jatropha curcas* E *Jatropha cinerea*

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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.*

accumulation (Sultana *et al*., 1999). In saline affected soils, Na^+ and Cl^- accumulation in leaf tissues causes necrosis of older leaves and may induce defoliation (Tester and Davenport, 2003). Furthermore, external apoplastic levels of Na^+ induce a deficiency of essential cations (K^+, Ca^{2+} and Mg^{2+}) by competition with Na^+ (Niu *et al*., 1995; Song and Fujiyama, 1996; Blumwald, 2000). Therefore, cation balance between Na^+ and essential cations in the plant can be used as indicators of nutritional homeostasis under high salt conditions (Kudo *et al*., 2010). Under salt stress, plants protect themselves controlling Na^+ and Cl^- uptake by changing their morphology and reducing transpiration by closing stomata (Hasegawa *et al*., 2000). To reduce salinity effects, plants also sequester Na^+ into vacuoles, adjust osmotic potential and regulate salt distribution from the roots to shoots (Munns, 2005).

Jatropha curcas ('Barbados nut', 'physic nut') is a tropical and subtropical perennial succulent shrub that grows to 3-5m (Maes *et al*., 2009) in Mexico and Central America (Achten *et al*., 2008), where annual precipitation is of 500-1000mm (Heller, 1996). *J. curcas* receives a lot of attention as a source of renewable energy from its oily (27-40%) seeds, which are easily converted into biodiesel that meets American and European standards (Achten *et al*., 2007). Francis *et al*. (2005) report that this species has drought, salinity and pest resistance, so it can grow in areas which are not suitable for most agriculturally important plants; however, abiotic stress factors reduce seed production from 12 to 0.4t·ha^{-1} (Achten *et al*., 2008). There are few studies about the effect of abiotic stress on *J. curcas* (Fairless, 2007), and it is not clear how salt stress affects its biomass productivity.

The arid northwestern area of Mexico has a dry subtropical climate (annual precipitation <200mm) with frequent long dry periods. Here, *Jatropha cinerea* ('Arizona nettlespurge', 'ashy jatropha', 'ashy limberbush', 'lomboy'), a wild species related to *J. curcas*, grows on saline soils along the coast and rocky areas. *J. cinerea* can withstand long droughts and flowers during the rainy season (June to October). Its succulent and bark have been used in traditional medicine.

In this study, the effects of salt stress on growth, water potential and ion content of young *J. curcas* plants were measured to gain basic information to assess its capacity to grow in saline soil, and compared to those on *J. cinerea*, a species that is adapted to dry and saline soils.

Materials and Methods

Plant material and growth conditions

Seeds of *Jatropha curcas* from Papantla, Veracruz, Mexico, and seeds 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 H_2SO_4 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-173, 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 at14 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

$$WC(\%) = [(FW-DW)/FW] \times 100$$

where WC: water content,

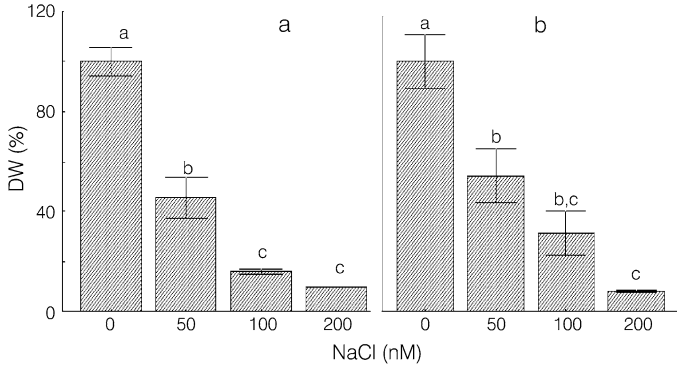

Figure1. Effect of NaCl on dry weight (DW) of *J. curcas* (a) and *J. cinerea* (b) grown at 0, 50, 100, and 200mM NaCl for 28 days. The control is shown as 100%. The same letters above each bar indicate no significant difference (Tukey test after ANOVA; P<0.05). Values are mean of three plants ±SE.

TABLE I
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

NaCl (mM)	DW (g)			S/R	Leaf area (cm^2)	Leaf number
	Root	Stem	Leaf			
Jatropha curcas						
0	0.79 ±0.08 a	2.90 ±0.56 a	1.83 ±0.04 a	6.0 ±1.3 †	425.7 ±6.2 a	11 ±1 a
50	0.41 ±0.08 b	1.40 ±0.66 b	0.70 ±0.10 b	5.1 ±0.8 †	205.0 ±3.4 b	7 ±0 b
100	0.18 ±0.03 c	0.37 ±0.03 b	0.33 ±0.07 c	3.9 ±0.5 †	86.2 ±20.7 c	6 ±1 b
200	0.11 ±0.03 c	0.27 ±0.03 b	0.15 ±0.02 d	3.8 ±0.9 †	48.4 ±1.2 c	4 ±1 b
Jatropha cinerea						
0	0.48 ±0.19 a	2.55 ±0.39 a	1.09 ±0.27 a	7.6 ±2.0 a	284.9 ±61.2 a	7 ±2 a
50	0.31 ±0.10 a,b	1.50 ±0.52 b	0.44 ±0.18 b	6.3 ±0.9 a,b	116.8 ±10.1 b	5 ±0 a
100	0.23 ±0.13 a,b	0.78 ±0.29 b,c	0.29 ±0.21 b	4.7 ±1.3 a,b	71.9 ±51.1 b	4 ±2 a,b
200	0.06 ±0.01 b	0.23 ±0.03 c	0.04 ±0.01 b	4.7 ±1.4 b	20.7 ±11.4 b	3 ±1 b

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.

DW: total dry weight, and FW: total fresh weight.

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 (g_s) 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 zipped 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^+, Ca^{2+} and Mg^{2+} were determined by atomic absorption spectrophotometry (AA660, Shimazu, Kyoto, Japan) after digestion using three acids (H_2SO_4:HNO_3:$HClO_4$; ratio of 1:4:10). Chlorine (Cl^-) was extracted in boiling water and the concentration was determined by ion chromatography (HIC−6A, Shimazdu, Kyoto, Japan).

Tissue solute concentrations were converted to osmolality based on tissue water content. The osmotic potential (Ψ_s) of Na^+, K^+, Ca^{2+}, and Mg^{2+} was calculated according to the van't Hoff equation Ψ_s= -nRT/V, where n: number of solute molecules, R: universal gas constant, T: temperature in ºK, 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.

Salinity effect on stomatal conductance, transpiration, leaf water potential, and total water content

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.

Stomatal conductance and water content was found to be related with dry weight and Na^+ uptake in both species ($P < 0.001$ in *J. curcas*; $P<0.05$ in *J. cinerea*; Table II). A strong correlation between dry weight and water potential was observed only in *J. cinerea* ($P<0.01$).

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).

Cl^- concentration also increased with increasing NaCl concentration in both species (Table III). However, it was difficult to obtain enough biomass to measure Cl^- at the higher NaCl treatments because of biomass reduction at these salinity levels.

The concentration of K^+ and Ca^{2+} decreased relative to the control, and Mg^{2+} concentration was affected only in the root under salinity in both species (Table III). Comparing the two species, K^+ and Mg^{2+} concentrations of *J. curcas* were higher than those of *J. cinerea* in shoot and root, and Ca^{2+} of *J. curcas* was higher in shoot but not in root.

Cation imbalance [Na^+ / (K^+ + Ca^{2+} + Mg^{2+})] 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

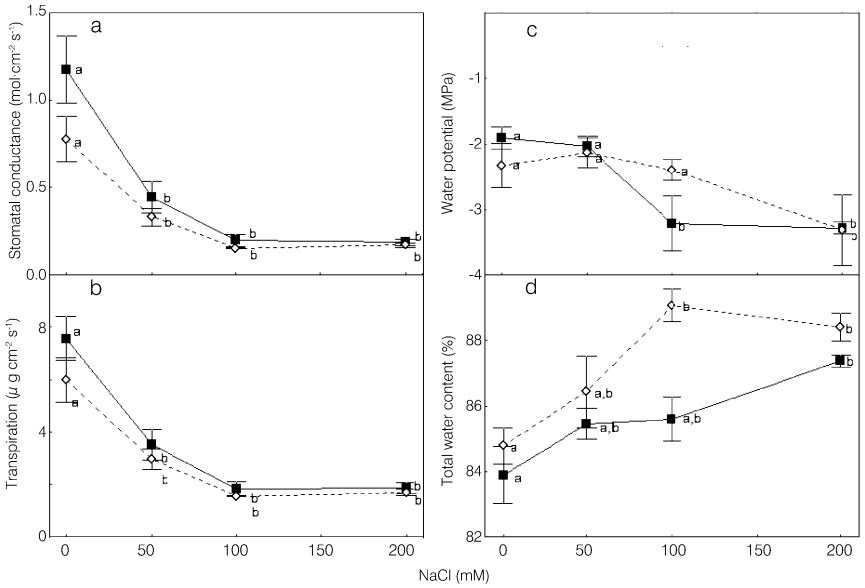

Figure 2. Effect of NaCl on stomatal conductance (a), transpiration (b), leaf water potential (c), and total water content (d) in plants of *J. curcas* (◇) and *J. cinerea* (■) grown at 0, 50, 100, and 200mM NaCl. Stomatal conductance, transpiration, and water potential were measured at 14 days after start of treatment, and water content was obtained at 28 days of treatment. The same letters indicate no significant difference (Tukey test after ANOVA; $P<0.05$). Values are mean of three plants ±SE.

TABLE II
CORRELATION COEFFICIENTS OF THE MEASURED PARAMETERS IN *J. curcas* AND *J. cinerea*

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

*, **, *** denote significance at P= 0.05, 0.01, and 0.001, respectively.

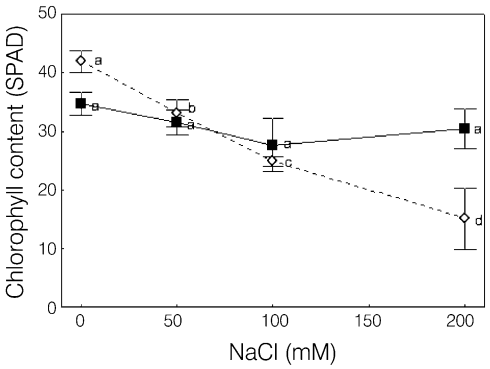

Figure 3. Effect of NaCl on chlorophyll content (SPAD value) in plant of *J. curcas* (◇◊) and *J. cinerea* (■) grown at 0, 50, 100, and 200mM NaCl for 28 days. The same letters indicate no significant difference (Tukey test after ANOVA, P<0.05). Values are mean of three plants ±SE.

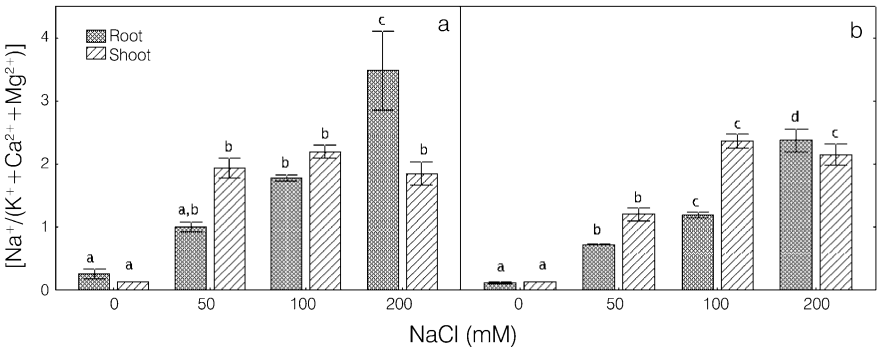

Figure 4. Effect of NaCl on cation imbalance of [Na^+ / (K^++Ca^{2+}+Mg^{2+})] in plants of *J. curcas* (a) and *J. Cinerea* (b) grown at 0, 50, 100, and 200mM NaCl for 28 days. The same letters above each bar indicate no significant difference (Tukey test after ANOVA, P<0.05). Values are mean of three plants ±SE.

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. curcas* declined more in shoot compared with *J. cinerea* by salinity treatment. In both species, Ψs of Na^+ were similar in root, and Ψs of Ca^{2+} and Mg^{2+} 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 Bjorkman, 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 CO_2 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 f 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

TABLE III
EFFECT OF NaCl ON MINERAL COMPOSITION (Na^+, Cl^-, K^+, Ca^{2+}, AND Mg^{2+}) 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

NaCl (mM)	Na^+ (mmol·g^{-1})		Cl^- (mmol·g^{-1})		K^+ (mmol·g^{-1})		Ca^{2+} (mmol·g^{-1})		Mg^{2+} (mmol·g^{-1})		Na^+ S/R
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	
Jatropha curcas											
0	0.19 ±0.01 c	0.15 ±0.02 d	0.06 ±0.02 b	0.12 ±0.04 b	0.94 ±0.09 a	0.78 ±0.07 a	0.32 ±0.06 a	0.23 ±0.05 a	0.21 ±0.01 †	0.34 ±0.11 a	1.3 ±0.2 b
50	1.21 ±0.24 b	0.78 ±0.05 c	0.27 ±0.05 b	0.30 ±0.03 a	0.68 ±0.11 b	0.58 ±0.05 a,b	0.22 ±0.01 b	0.21 ±0.04 a	0.14 ±0.01 †	0.29 ±0.02 b	1.6 ±0.2 a,b
100	2.43 ±0.07 a	1.19 ±0.01 b	0.82 ±0.20 a	n.d	0.63 ±0.03 b	0.51 ±0.04 b	0.23 ±0.01 b	0.18 ±0.04 a	0.16 ±0.01 †	0.31 ±0.02 a	2.0 ±0.1 a
200	2.29 ±0.24 a	1.54 ±0.41 a	n.d	n.d	0.67 ±0.04 b	0.37 ±0.07 b	0.22 ±0.01 b	0.10 ±0.04 b	0.18 ±0.02 †	0.17 ±0.03 b	1.5 ±0.4 a,b
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,c	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,b	1.2 ±0.2 a
100	1.34 ±0.16 a	1.16 ±0.03 a,b	0.64 ±0.09 a	0.40 ±0.09 a	0.36 ±0.07 b	0.31 ±0.02 b,c	0.15 ±0.04 b	0.15 ±0.02 b	0.09 ±0.02 †	0.20 ±0.02 a,b	1.2 ±0.2 a
200	1.28 ±0.28 a	1.58 ±0.18 a	n.d	n.d	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,b

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.

TABLE IV
EFFECT OF NaCl ON INORGANIC ION OSMOTIC POTENTIAL (Ψ_s) IN SHOOTS AND ROOTS OF *J. curcas* AND *J. cinerea* GROWN AT 0, 50, 100, AND 200mM NaCl FOR 28 DAYS

NaCl (mM)	Na^+ Ψ_s (MPa)		K^+ Ψ_s (MPa)		Ca^{2+} Ψ_s (MPa)		Mg^{2+} Ψ_s (MPa)	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Jatropha curcas								
0	0.07 ±0.00 c	0.08 ±0.04 c	0.41 ±0.03 a	0.17 ±0.04 a	0.10 ±0.00 a	0.09 ±0.02 a	0.07 ±0.00 a	0.07 ±0.03 †
50	0.50 ±0.04 a,b	0.22 ±0.04 b,c	0.16 ±0.04 b	0.12 ±0.02 a,b	0.06 ±0.00 b	0.05 ±0.01 a,b	0.04 ±0.01 b	0.05 ±0.01 †
100	0.60 ±0.00 a	0.22 ±0.08 a,b	0.17 ±0.02 b	0.10 ±0.03 a,b	0.07 ±0.01 b	0.04 ±0.00 b	0.04 ±0.00 b	0.06 ±0.01 †
200	0.40 ±0.08 b	0.36 ±0.18 a	0.15 ±0.01 b	0.09 ±0.03 b	0.03 ±0.00 c	0.05 ±0.02 a,b	0.03 ±0.01 b	0.03 ±0.01 †
Jatropha cinerea								
0	0.10 ±0.01 c	0.03 ±0.00 c	0.48 ±0.02 a	0.16 ±0.02 a	0.17 ±0.02 a	0.05 ±0.01 a	0.11 ±0.02 a	0.07 ±0.02 a
50	0.58 ±0.06 b	0.16 ±0.07 b	0.31 ±0.002 b	0.12 ±0.01 b	0.10 ±0.01 b	0.04 ±0.01 a,b	0.07 ±0.00 b	0.06 ±0.01 a,b
100	0.84 ±0.02 a	0.25 ±0.03 b	0.22 ±0.04 c	0.10 ±0.01 b	0.08 ±0.02 b	0.04 ±0.00 a,b	0.05 ±0.01 b	0.06 ±0.01 a,b
200	0.81 ±0.14 a	0.36 ±0.07 a	0.24 ±0.03 c	0.09 ±0.02 b	0.08 ±0.01 b	0.03 ±0.00 b	0.06 ±0.00 b	0.04 ±0.00 b

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.

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 Ca^{2+} and K^+ specific functions such as cellular expansion and enzyme activation for adequate growth (Song and Fujiyama, 1996; Nieves-Cordones *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 and1.19 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 Ca^{2+} 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 Ca^{2+} 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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