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Physiological and morphological responses to flooding with fresh or saline water in *Jatropha curcas*

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**Abstract**

*Jatropha curcas* L. has recently drawn the attention of the international research community due to its potential as a biodiesel crop. Although *Jatropha* is relatively tolerant to drought and salinity, poorly drained production areas have suffered increasing problems associated with flooding by saline water. Since the combined effects of flooding and salinity stress are not known, the objective in this work was to investigate physiological and morphological responses of *J. curcas* plants to flooding by fresh water or salt water. Plant growth, leaf water relations, leaf gas exchange, chlorophyll fluorescence parameters, carbohydrate and organic solutes, and leaf mineral nutrients were determined in three month-old plants exposed to flooding for 10 days with water containing 0, 150 or 300 mM NaCl. Flooding decreased shoot and root growth but there were no significant difference between 0 and 300 mM NaCl treatments. In 0 mM NaCl flooded plants, leaves suffered dehydration as relative water content (RWC) and leaf water potential (Ψ<sub>Ψ</sub>) decreased progressively resulting in decreased leaf turgor potential (Ψ<sub>T</sub>). In 300 mM NaCl flooded plants, however, Ψ<sub>Ψ</sub> remained high as leaf osmotic potential (Ψ<sub>Ψ</sub>) decreased and Ψ<sub>P</sub> increased. Increased leaf Cl− and Na+ concentration enabled this osmotic adjustment avoiding leaf dehydration but toxic values were reached in leaves. Leaf gas exchange parameters (net assimilation of CO<sub>2</sub> (A<sub>CO</sub>), stomatal conductance (g<sub>st</sub>)) and quantum efficiency of PSII (Φ<sub>PSII</sub>) were reduced similarly by flooding but internal CO<sub>2</sub> concentration was highest in the 300 mM NaCl treatment. Soluble sugars and starch were reduced in leaves and roots, and leaf Ca, Mg and P concentration was increased in plants under flooding. *J. curcas* plants are sensitive to flooding condition regardless of the level of salinity confirming their salinity tolerance even under flooded conditions.

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1. Introduction

Soil waterlogging affects worldwide ecosystem conservation and agricultural production (McFarlane and Williamson, 2002; Upadhyay and Panda, 2005) especially in areas with poorly drained soils (Kijne, 2006). In coastal areas, saltwater flooding can occur from hurricanes (Albrigo et al., 2005) and saline intrusion from the sea (Bianchette et al., 2009). Complete O<sub>2</sub> depletion from a flooded soil can occur in few days resulting in a decrease in soil redox potential to levels about −250 mV, negatively affecting the performance of the crops (Syvertsen et al., 1983) physiological responses (Arbona et al., 2008) and growth (Wang and Jiang, 2007).

Since waterlogging solubilizes soil solutes, flooding often occurs in conjunction with salinity stress (Bianchette et al., 2009). Although there have been many studies on physiological responses of crops to high salinity (Levy and Syvertsen, 2004) and to flooding (Jackson and Colmer, 2005), few investigations have studied interactions between flooding and salinity stress at the same time (Naumann et al., 2008; Song et al., 2011).

Salinity and flooding can also cause nutrient deficiencies or imbalances in the plant tissue. It has been observed in plants under salinity condition that the competition of Na<sup>+</sup> and Cl<sup>−</sup> with nutrients caused a decrease in K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, NO<sub>3</sub><sup>−</sup> concentration (Romero-Aranda et al., 1998). Flooding can decrease nutrient uptake in plants, but generally Fe and Mn uptake significantly increases with flooding (Jones, 1972) since reduced from of these nutrients are more available as a consequence of increasing of redox
potential produced by low oxygen values in the soil (Waldren et al., 1987).

It is difficult to quantify the combined effects of flooding and salinity because osmotic effects of salinity under flooded conditions (Song et al., 2011), can differ from those under dehydrating conditions (Pérez-Pérez et al., 2007). Growth reduction from flooding with saline water can be greater than flooding with fresh water since flooding with saline water can cause an initial rapid increase of Cl− and Na+ transport to the shoot (Craig et al., 1990).

Factors influencing tolerance to flooding can include reopening of stomata after soil flooding and a rapid recovery of leaf conductance after flooding (Li et al., 2004) but ACO2 and gs can be independent during and after flooding (Fernandez et al., 1999). High photosynthetic rates during soil flooding (Li et al., 2007) can lead to high starch content in roots (Gravatt and Kirby, 1998), and leaves (Laan and Blom, 1990).

J. curcas (purge nut/phytic nut) belongs to the family Euphorbiaceae and is widely distributed throughout many tropical and sub-tropical regions throughout America, Africa and Asia (Takeda, 1982). This plant is characterized by its high drought and salinity tolerance (Maes et al., 2009; Silva et al., 2010), high oil content, rapid growth and adaptability to widely different agro-climatic conditions (Divakara et al., 2010). J. curcas seeds have received much attention because their oil can be converted in biodiesel (Openshaw, 2000) and because J. curcas can improve soil resistance to wind erosion and water erosion under flooded conditions (Ogunwole et al., 2008). The tolerance of J. curcas flooding especially under saline conditions has not been described. The objective of this research was to explore the physiological and morphological responses of J. curcas plants under non-saline and saline flooded conditions.

2. Materials and methods

2.1. Plant material and experimental condition

Seeds of J. curcas L. were germinated in trays of sterilized vermiculite wetted with 0.5 mmol/L CaSO4 in the dark at 29°C. Four days after germination the seedlings were transferred to 25-L plastic pots containing a soilless substrate of Canadian blond peat moss blended with fiber of coconut and perlite (Compost Reciclable S.L., Murcia, Spain). Plants were grown in a greenhouse under maximum photosynthetically active radiation (PAR) of 1000 μmol m−2 s−1, day/night temperature of 35/18±3°C, a day/night relative humidity of 55/75±5% and a natural 16-h photoperiod. In the non-flooding control (Drained) treatment, irrigation was carried out. Plants were irrigated using a drip system and a complete nutrient solution of 7.75 mM NO3−, 0.7 mM H2PO4−, 4.05 mM K+, 2.20 mM Ca2+, 0.5 mM Mg2+, 0.5 mM SO42− and 0.6 mM Fe. Plants were watered every day with the amount sufficient of nutrient solution to leach from the bottom of all pots. Flooding treatments were begun when the plants were three month-old by submerging each pot into a 40L undrained pot filled with tap water containing either 0 (F0), 150 (F150) or 300 (F300) mM NaCl. The water level was maintained at 4 cm above soil surface for 9 days. All fertilizer application was ceased during flooding treatments while control plants were irrigated with tap water and drained. Tap water contained (mM): 8.86 SO42−, 1.16 HCO3−, 3 Cl−, 3.5 K+, 1.2 Ca2+, 2.22 Mg2+, 4.13 Na+. For each treatment, six replicate plants were studied.

2.2. Leaf water relations

Pre-dawn leaf water potential (Ψ0) was measured with a Scholander-type pressure chamber (PMS instrument, Corvallis, OR; Scholander et al., 1965) using a single mature leaf in the mid-stem region of each of the six replicate plants. After Ψ0 was measured, leaves were immediately wrapped tightly in aluminum foil, frozen by immersing in liquid nitrogen and subsequently stored in air-tight plastic bags at −18°C. After thawing, Ψw of the extracted sap was measured at 25±1°C with an osmometer (Digital Osmometer, Wescor, Logan, UT). Turgor potential was (Ψp) calculated as the differences between pre-dawn Ψw and Ψw. For determination of relative water content (RWC), leaf discs from adjacent leaves were weighed to obtain a fresh mass (Mf) and submerged in a beaker of water overnight in the dark so leaf discs could become fully hydrated. Discs were reweighed to obtain turgid mass (Mt) and dried at 80°C for 24 h to obtain dry mass (Md). RWC was calculated as ([Mf − Md]/[Mf − Mt]) × 100 according to Morgan (1984). Leaf osmotic potential at full turgor (Ψw(0)H2O) was measured on one leaf per plant after full hydration overnight as above. Fully turgid leaves were then frozen in liquid nitrogen, and Ψw(0)H2O was measured as above.

2.3. Leaf gas exchange, and chlorophyll fluorescence parameters

Net assimilation of CO2 (ACO2), stomatal conductance (gs), instantaneous leaf water use efficiency (WUE = ACO2/leaf transpiration) and ratio of ambient to intercellular CO2 (Ci/Ca) were measured using a portable photosynthesis system (model LCA-4, ADC Bioscientific Ltd., Hoddesdon, UK) with a leaf chamber PLC-4N (11.35 cm2), configured in an open system. During all measurements, PAR exceeded 800 μmol m−2 s−1, leaf temperature was 30±2°C and leaf-to-air vapor pressure difference was 2.4±0.4 kPa within the cuvette. Chlorophyll fluorescence parameters were also measured on similar leaves as those used for gas exchange parameters with pulse-modulated fluorometer (model FMS2, Hansatech, King’s Lynn, Norfolk, UK) using standard instrument settings (saturating pulse of 12,000 μmol m−2 s−1) with additional far red light (735 nm) to estimate ground state fluorescence (F0). In 30 min dark-adapted leaves were measured F0, minimal fluorescence in the dark-adapted state, and Fm (maximal fluorescence in the dark-adapted state after a short light-saturation light), and used to estimate the maximum quantum yield of PSII Fv/Fm = (Fm − F0)/Fm (Genty et al., 1989). The minimal fluorescence in the light-adapted state (Fv) and the maximal fluorescence (Fm) was measured in light-adapted leaves, and value when all reaction centers are closed after a pulse of saturating light obtained by temporarily turning the light off to drain the electrons from PSII, and the steady state fluorescence yield (Fv). The energy harvesting efficiency of PSII in a light-adapted leaf was, thus, calculated as: Fv/Fm = (Fm − F0)/Fm, quantum efficiency of PSII as: ΦPSII = (Fv/F0)/Fm, and photochemical quenching (qP) as: qP = (Fm − Fv)/(Fm − F0) (Genty et al., 1989).

2.4. Growth and nutrient concentration

At the end of the experiment, the height of seedling was measured, plants were harvested and leaves, stem and root weighed. Tissues were briefly rinsed with deionised water, oven-dried at 60°C for at least 48 h, weighed and ground to a fine powder. Dry weight (DW) of leaves, stem and roots were used to calculate total dry biomass and shoot to root DW ratio. Mineral concentration (Na+, Ca2+, K+, Mg2+, P and S) in the different of plant tissues were determined by inductively coupled plasma emission optical spectrometry (Iris Intrepid II, Thermo Electron Corporation, Franklin, USA) after previous acid digestion in HNO3: H2O2 (5:3 by volume) in a microwave reaching 190°C in 20 min and holding at this temperature during 2 h (CEM Mars Xpress, North Carolina, USA). Tissue Cl− concentration was measured with a
silver ion titration chloridometer (Corning 926, Sherwood, UK) previous extraction with deionized water (Garcia-Sánchez et al., 2002).

2.5. Carbohydrates, quaternary ammonium compounds

Extraction of carbohydrates in leaves, stem and root tissue at the end of experimental period was carried out using 80% ethanol in constant mixing for 4 h at 17–24 °C. A sulfuric acid assay with anthrone reagent was conducted to measure total soluble carbohydrates (Hodge and Hofreiter, 1962) and the reducing sugars concentration was determined according to the Nelson–Somogyi method (Nelson, 1944; Somogyi, 1952). The procedure used for measurement of starch concentration included the extraction from the pellet with MES solution and gelatinization with heat-stable alpha amylase in a 1-h 92–93 °C water bath (Haisig and Dickson, 1979). Both soluble sugar and starch were quantified using glucose as standard. Quaternary ammonium compounds (QAC) were extracted from fresh tissues with 1 M H₂SO₄ and quantified using a glycine–betaine standard according to the method described in Grieve and Grattan (1983).

2.6. Statistical analysis

Statistical analyses included analysis of variance (ANOVA) using the SPSS 19.0 statistical package (IBM, Chicago, IL). When the treatment effects were significant (P < 0.05), treatment means were separated using Tukey’s multiple range test.

3. Results

3.1. Plant growth and symptoms

All flooding treatments, regarding the NaCl concentration, decreased leaf dry weight (DW) by about 62% while root DW was decreased by about 38% compared to non-flooded plants (Fig. 1). Stem DW was reduced by 28% but only in the freshwater flooding treatment. Shoot to root DW ratio and plant height were not affected by any flooding treatment. Flooded plants lost about half their leaves (data not shown). The fallen leaves from the freshwater flooded plants, showed symptoms of dehydration and necrotic areas, whereas the leaves fallen from salinized flooding treatments F150 and F300 appeared healthy without visible damage symptoms (Fig. 7).
3.2. Leaf water relation

One day after starting the flooding treatments, there were no significant effects on leaf water relation parameters (Fig. 2). After day 4, the $\Psi_w$ and $\Psi_p$ of plants under freshwater flooding treatment decreased progressively through time but $\Psi_p$ was not affected during the 9 days. $\Psi_w$ of plants from saltwater flooding treatment F150 was not affected but at the end of the experimental period, the $\Psi_p$ was reduced and $\Psi_p$ was increased. The $\Psi_w$ of F300 plants was unchanged until the end of the experiment but $\Psi_p$ decreased and $\Psi_p$ increased since day 4.

At the end of the experimental period, leaf RWC was lower in freshwater flooded plants than in the control plants (Fig. 3). The F300 treatments, however, increased the leaf RWC but not in the F150 plants. Leaf osmotic potential at full turgor in F300 plants was significantly lower than control treatments.

3.3. Leaf gas exchange and chlorophyll fluorescence parameters

Net CO$_2$ assimilation rate ($A_{\text{CO}_2}$), $g_s$ and leaf WUE were reduced similarly over time in all three flooding treatments compared to control plants (Fig. 4). There were no significant differences among the different flooding treatments except on day 3 when $A_{\text{CO}_2}$ was lower for F300 plants than for F150 plants. The $C_i/C_a$ ratio tended to increase with flooding over time and was highest for the F300 treatment. Within the freshwater flooded plants, there was a significant negative relationship between $A_{\text{CO}_2}$ and stomatal conductance ($R^2 = 0.74$). The $A_{\text{CO}_2}$ values for plants under saltwater flooding treatment were higher than those of freshwater flooding treatment (Fig. 5). However, in $F_v/F_m$ of plants from F0 and F300 had values higher than plants from F150 treatment. F300 plants had the lowest $F_v/F_m$ at 7 and 10 days after flooding.

3.4. Mineral concentration in plant tissues

Chloride and Na$^+$ concentrations increased in all plant tissues with increasing NaCl concentration in the saltwater flooding treatments in all plant tissues and the Cl$^-$ concentration was higher than the Na$^+$ concentration (Fig. 6). Highest Cl$^-$ and Na$^+$ concentrations were observed in the leaves, whereas roots and stem had similar values.

Leaf Ca$^{2+}$ concentration increased in the F150 treatment and root Ca increased F300 compared with the control treatment (Table 1), Root K$^+$ concentration decreased in both F150 and F300 treatments. Leaf Mg$^{2+}$ concentration increased in the F0 and F150 treatments and in the stem for the F300 treatment while root Mg$^{2+}$ concentration decreased for both saltwater flooding treatments. Leaf P concentration and root S concentration significantly increased for the F0 and F300 treatments but not for the F150 treatment.

3.5. Carbohydrates and QAC concentration in plant tissues

The three flooding treatments significantly decreased the total soluble sugars concentration in leaves and roots, while it was only reduced by F300 treatment in the stem (Table 2). The concentration of reducing sugars in the leaves was only reduced in the F300 plants whereas the concentration in the stem was reduced in the F150 and F300. All three flooding treatments decreased reducing sugars in roots and starch in leaves, The QAC compounds concentration was only decreased by the F300 treatment compared to the control plants.

![Table 1](image_url)

Table 1: Concentration of Ca$^{2+}$, K$^+$, Mg$^{2+}$, P and S in leaves, stems and roots in J. curcus seedlings subjected to flooding with fresh water (F), 150 mM NaCl (F150), 300 mM NaCl (F300) or non-flooded control treatments for 10 days. Values are the mean of 6 samples.

<table>
<thead>
<tr>
<th></th>
<th>Drained</th>
<th>F0</th>
<th>F150</th>
<th>F300</th>
<th>P (%)</th>
<th>S (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flooding</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Ca$^{2+}$ (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drained</td>
<td>2.10 b</td>
<td>3.10</td>
<td>0.69 b</td>
<td>0.41 c</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>F0</td>
<td>2.27 ab</td>
<td>3.47</td>
<td>0.85 a</td>
<td>0.60 a</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Leaf</td>
<td>2.55 a</td>
<td>3.06</td>
<td>0.86 a</td>
<td>0.46 bc</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>F300</td>
<td>1.99 b</td>
<td>3.23</td>
<td>0.69 b</td>
<td>0.57 ab</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>Root</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drained</td>
<td>0.94</td>
<td>2.54</td>
<td>0.24 b</td>
<td>0.29</td>
<td>0.07 b</td>
<td></td>
</tr>
<tr>
<td>F0</td>
<td>0.97</td>
<td>2.75</td>
<td>0.26 b</td>
<td>0.34</td>
<td>0.08 b</td>
<td></td>
</tr>
<tr>
<td>Leaf</td>
<td>1.04</td>
<td>2.81</td>
<td>0.25 b</td>
<td>0.31</td>
<td>0.12 a</td>
<td></td>
</tr>
<tr>
<td>F300</td>
<td>1.15</td>
<td>2.83</td>
<td>0.35 a</td>
<td>0.36</td>
<td>0.12 a</td>
<td></td>
</tr>
</tbody>
</table>
| Stomatal conductance parameters (Fig. 2). After day 4, the $\Psi_w$ and $\Psi_p$ of plants under freshwater flooding treatment decreased progressively through time but $\Psi_p$ was not affected during the 9 days. $\Psi_w$ of plants from saltwater flooding treatment F150 was not affected but at the end of the experimental period, the $\Psi_p$ was reduced and $\Psi_p$ was increased. The $\Psi_w$ of F300 plants was unchanged until the end of the experiment but $\Psi_p$ decreased and $\Psi_p$ increased since day 4.
4. Discussion

4.1. Plant growth

J. curcas should be considered a flood-sensitive species because as little as 10 days under flooding condition caused a 30% reduction in the total biomass compared to non-flooded plants. In general, root submergence of other species that are not tolerant to flooding for 10–20 days is critical for reducing total biomass from 30 to 50% (Striker et al., 2007). Soil flooding similarly reduced leaf and root biomass in J. curcas plants such that shoot to root ratio was not affected. Flooding-induced leaf area reduction was due mainly to the premature senescence and leaf abscission rather than to a reduction in leaf growth or inhibition of leaf expansion as a result of decreases in cell wall extensibility (Smit et al., 1989). The root reduction effect is a typical response of flooding-sensitive plants and could be due to inhibiting root formation and branching, growth of existing roots and mycorrhizae, and/or by inducing root loss from decay (Kozlowski, 1984; Kozlowski and Pallardy, 1997). Different growth responses to short-term (10 days duration) between freshwater and saltwater flooding were only observed in the visual symptoms of the defoliated leaves as the non-salinized

![Graph showing the effect of flooding treatments on RWC and Ψπ100.](image)

**Fig. 3.** Effects of 10 days soil flooding with fresh water (F0), 150 mM NaCl (F150), 300 mM NaCl (F300) or non-flooded (Drained) control treatments on leaf osmotic potential at full turgor (Ψπ100) and leaf relative water content (RWC) in *Jatropha curcas* seedlings. Bars represent means ± S.E (n=6). * and ** indicate significant differences at P<0.05 and 0.01, respectively. Means followed by different letters are significantly different (P<0.05) according to Tukey’s multiple range tests.

![Graph showing the effect of flooding treatments on CO2 assimilation, stomatal conductance, WUE, and leaf Na+/Ca+ ratio.](image)

**Fig. 4.** Net assimilation of CO2 (Aco2), stomatal conductance (gs), instantaneous leaf water use efficiency (WUE = Aco2/leaf transpiration), CO2 ambient concentration: CO2 substomatal concentration ratio (Ci/Ca) in *Jatropha curcas* seedlings at 1, 4, 7 and 10 days after starting the flooding with fresh water (F0), 150 mM NaCl (F150), 300 mM NaCl (F300) or non-flooded control (Drained) treatments. ns indicates non-significant differences Bars represent means ± S.E (n=6). * , ** and *** indicate significant differences at P<0.05, 0.01 and 0.001, respectively. Means followed by different letters are significantly different (P<0.05) according to Tukey’s multiple range tests.
flooded leaves had symptoms of desiccation and necrotic areas, while leaves dropped from salinized flooded plants appeared relatively healthy (Fig. 7). This implied that the cause of leaf loss in freshwater and saltwater flooding was different as supported by the water relations data.

4.2. Leaf water relation

Freshwater flooded *J. curcas* plants had leaf water relation responses similar to other flooding-sensitive plant species such as *Lotus tenuis* (Striker et al., 2007) and *Capsicum annum* (Razi and Davies, 1997) where the leaf water potential ($\Psi_w$) decreases with duration of flooding. In our experiment, this reduction was due to a decreasing in the leaf turgor potential ($\Psi_T$) and RWC, that plants of *J. curcas* under flooding condition had a reduced ability to take up water causing resulting in dehydration (Nicolás et al., 2005). A molecular basis to explain the mechanisms by which root permeability to water is regulated in response to anoxia has been related to a decrease in pH, an early cellular response to oxygen deprivation, which regulated root water transport during anoxic stress through the gating of aquaporins (Tournaire-Roux et al., 2003). Other plant species as *Solanum lycopersicum*, also reduced $\Psi_w$ after 2–6 h of

![Fig. 5. Quantum efficiency ($\Phi_{psii}$), photochemical quenching ($qP$), harvesting efficiency ($F_v/F_m$) and maximum quantum yield ($F_v/F_m$) of PSII in Jatropha curcas seedlings at 1, 4, 7 and 10 days after starting the flooding with fresh water (F0), 150 mM NaCl (F150), 300 mM NaCl (F300) or non-flooded control (Drained) treatments. Bars represent means ± S.E. (n=6). *, ** and *** indicate significant differences at P < 0.05, 0.01 and 0.001, respectively. ns indicates non-significant differences. Means followed by different letters are significantly different (P<0.05) according to Tukey’s multiple range tests.](#)

| Table 2 | Concentration of total soluble sugars, reducing sugars, starch and Quaternary ammonium compounds (QAC) on leaves, stem and root in *J. curcas* seedlings subjected to flooding with fresh water (F), 150 mM NaCl (F150), 300 mM NaCl (F300) or non-flooded control treatments for 10 days. Values are the mean of 6 samples. |
|------------------------|------------------------|------------------------|------------------------|------------------------|
| Flooding               | Solubles sugar (µg gluc mg⁻¹ d.w.) | Reducing sugars (µg gluc mg⁻¹ d.w.) | Starch (µg gluc mg⁻¹ d.w.) | QAC (µg mg⁻¹ d.w.) |
| Drained                | 58.9 a                 | 7.2 b                   | 34.8 a                 | 39.7                   |
| F0                     | 26.3 b                 | 5.3 b                   | 16.5 b                 | 34.2                   |
| F150                   | 27.5 b                 | 4.9 b                   | 20.0 b                 | 49.1                   |
| F300                   | 31.0 b                 | 10.1 a                  | 15.4 b                 | 42.8                   |
| Drained                | 148.6 a                | 16.0 a                  | 150.9 a                | 41.0                   |
| F0                     | 120.8 a                | 14.0 ab                 | 84.8 b                 | 54.7                   |
| F150                   | 153.2 a                | 8.9 c                   | 127.8 ab               | 43.6                   |
| F300                   | 83.1 b                 | 10.9 bc                 | 152.4 a                | 46.0                   |
| Drained                | 112.2 a                | 18.1 a                  | 31.0 a                 | 43.2 a                 |
| F0                     | 29.0 bc                | 8.6 b                   | 17.4 b                 | 44.4 a                 |
| F150                   | 44.2 b                 | 7.9 b                   | 24.3 ab                | 52.4 a                 |
| F300                   | 18.8 c                 | 6.3 b                   | 6.6 c                  | 31.1 b                 |

ns indicates non-significant differences. Means followed by different letters are significantly different (P<0.05) according to Tukey’s multiple range tests.

* Indicates significant differences at P<0.05.

** Indicates significant differences at P<0.01.

*** Indicates significant differences at P<0.001.
starting flooding, however, in the following day the $\Psi_w$ was again increased to values similar to the well-drained plants due to stomatal closure avoiding leaf dehydration (Else et al., 1996).

The combination of NaCl and flooding (F150 and F300 treatment) in J. curcas plants avoided leaf tissue dehydration as leaf relative water content and leaf turgor pressure were similar to the control treatment, or even increased at the end of the experiment in F300 plants. Similarly, Slama et al. (2008) and Sucre and Suárez (2011) found that the presence of salt in the culture medium of plants subjected to drought stress prevented leaf tissue dehydration although the exact mechanism by which the plants maintained their water balance was not completely explained. In our experiment, data from leaf water relations and leaf gas exchange parameter suggest that the increased Cl$^-$ and Na$^+$ in the leaves decreased the $\Psi_w$, and this was accompanied by a high flux of entering water to the cells increasing $\Psi_h$. This water in the leaf cells was not lost to transpiration since the high concentration of Cl$^-$ and Na$^+$ in the leaves caused stomatal closure (Silva et al., 2010). Thus, although the flooding limited the water entering in the root system, the saline condition caused stomatal closure and avoided a negative water balance. The $\Psi_w$ remained more or less stable throughout the experiment and leaf dehydration did not occur.

4.3. Leaf gas exchange and chlorophyll fluorescence

Stomatal closure is a common response to soil oxygen deficiency caused by flooding due to decreases in hydraulic conductivity (Syvertsen et al., 1983) and/or hormonal mechanisms involved (Else et al., 1996; Naumann et al., 2008; Horchani et al., 2010). The negative correlation between the $\Psi_w$ and stomatal conductance in freshwater flooded plants suggested that the partial stomatal closure was the result of a leaf water deficit, probably due to a reduction in root hydraulic conductance (Syvertsen et al., 1983; Davies and Flore, 1986). However, several other studies have shown that stomatal closure of flooded plants can be induced by hormonal signal transmitted from the roots to the shoots (Zhang and Davies, 1990; Else et al., 1996) or even by the loss of root signaling via cytokinin or gibberelin (Else et al., 2009). In our experiment we did not measure hormonal response of J. curcas plants but if flooding did induce hormonal signals in the roots, this was not an effective mechanism to avoid leaf dehydration. The stomatal closure in salt-water flooded plants was probably due to the toxic effect of Cl$^-$ and/or Na$^+$ rather flooding condition, as observed in other plant species (Naumann et al., 2007). The high concentration of these toxic ions in the leaves decreased the photosynthetic potential, as
Increases in proline and quaternary ammonium compounds are considered typical responses of plants exposed to salt and drought stress contributing to reduced leaf osmotic potential among other functions (Cha-um and Kirdmanee, 2009). However, in J. curcas plants, these organic solutes were not a key factor in the tolerance mechanisms to flooding condition since QAC's concentration were not changed.

4.5. Mineral concentration in the plant tissue

J. curcas plants are not able to restrict the uptake and/or transport of Cl\(^-\) or Na\(^+\) ions from roots to shoots as the highest accumulation was observed in the leaves. These plants accumulated more Cl\(^-\) than Na\(^+\) in their plant tissues unlike most salinized plant species that accumulate less Cl\(^-\) than Na\(^+\) (Munns and Tester, 2008). The high leaf Cl\(^-\) and/or Na\(^+\) concentrations determined the physiological response of these plants to the combinations of salt and flooding stress. High concentrations of these ions act as an osmoticum avoiding the leaf dehydration but their toxicity lead to a decrease in A\(_{CO_2}\). Similar results were found in J. curcas plants watered with 50 mM NaCl for 8 days (Silva et al., 2010). These authors observed that the high leaf Cl\(^-\) and/or Na\(^+\) concentration contributed to osmotic adjustment of the leaves avoiding the leaf dehydration, but A\(_{CO_2}\) was reduced a 82% relative to non-salinized plants.

It is well known that oxygen deficiency caused by soil flooding can decrease nutrient uptake in plants. Uptake of nutrients can depend on plant species and nutrient availability in soils, but generally Fe and Mn uptake significantly increases with flooding (Jones, 1972), and N, P and K can be reduced (Kozlowski, 1984; Pezeshki, 1995); Ca and Mg uptake can altered less than that of N, P or K by flooding (Kozlowski, 1984). In our study nutrient status of J. curcas plants was affected by the flooding treatments but this effect was probably not a limiting factor in the plant growth since the general trend was for increasing the leaf Ca, Mg and P concentration.

In summary, J. curcas plants should be considered as a flood-sensitive species under freshwater and saltwater condition since flooding for 10 days reduced the total plant dry weight in a 30%. Leaf gas exchange and chlorophyll fluorescence parameters showed a similar pattern for both freshwater and saltwater flooding conditions as A\(_{CO_2}\), gs, and \(\Phi_{PSII}\) were reduced in the three flooding treatments similarly by the end of the experimental period. However, the cause of this physiological response differed between freshwater or saltwater flooding treatments. In fresh water flooding, leaf water relations parameters suggest that leaf dehydration caused by decreased root hydraulic conductance reduced A\(_{CO_2}\). In saltwater flooding, the Cl\(^-\) and Na\(^+\) toxicity reduced A\(_{CO_2}\) and decreased carbohydrate concentrations in leaves and roots. Mineral nutrition was affected by the flooding conditions but this was apparently not a limiting factor in the plant growth reduction. J. curcas plants are sensitive to flooding regardless of the level of salinity confirming their salinity tolerance even under flooded conditions.

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References


