Salt stress induced damages on the photosynthesis of physic nut young plants

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ABSTRACT: Salinity is a major limiting factor to crop productivity in the world especially in semiarid regions. The aim of this study was to evaluate the photosynthetic resistance of Jatropha curcas (L.) young plants subjected to salt stress. The experiment was carried out in a completely randomized design with treatments in a 2 × 3 factorial: two NaCl levels (0 and 100 mmol L⁻¹) and three harvest times: 7 and 14 days of salt exposure and three days of recovery. Leaf Na⁺ and Cl⁻ concentrations and the K⁺/Na⁺ ratios, after seven days of salt exposure, did not reach ionic toxic levels, suggesting that the NaCl-induced osmotic effects prevailed over the ionic ones. Under this condition, the salt stress caused reduction in leaf gas exchange parameters, such as CO₂ fixation, stomatal conductance and transpiration. In contrast, salt stress did not change the photochemical efficiency of photosystem II. Conversely, after 14 days of treatment, Na⁺ and Cl⁻ ions reached very high concentrations, up to toxic levels in leaves. Under such conditions, both leaf gas exchange and photochemistry suffered strong impairment probably caused by ionic toxicity. The recovery treatment for 3d did not significantly decrease the leaf salt concentrations and no improvement was observed in the photosynthetic performance. Physic nut young plants are sensitive to high NaCl-salinity conditions, with high leaf Na⁺ and Cl⁻ concentrations, low K⁺/Na⁺ ratio and great photosynthetic damages due to stomatal and biochemical limitations.

Key words: Jatropha curcas, chlorophyll fluorescence, gas exchange, ionic toxicity, salinity

Introduction

Salinity adversely affects plant growth and development, with nearly 20% of the world cultivated area and about half of the world irrigated lands being affected by salt stress (Sairam and Tyagi, 2004). This problem is more relevant in semiarid regions with low rainfall and high evaporative demand, which strongly contribute to increase soil salinization (Viégas et al., 2001). In these regions, the problem of soil secondary salinization is exacerbated by the use of low quality water associated with inadequate techniques of soil management (Ferreira-Silva et al., 2009).

The accumulation of salt ions in plants can cause osmotic stress, ionic toxicity and induce nutritional deficiencies (Munns, 2002). When Na⁺ and Cl⁻ ions reach...
high concentrations in leaves, they cause impairment in both biochemical and photochemical processes of photosynthesis (Munns and Tester, 2008). In addition to these physiological disturbances, abiotic factors such as excess of the salts (Cavalcenti et al., 2007) and heavy metals can trigger secondary stresses as the oxidative stress due to accumulation of reactive oxygen species (Gratão et al., 2005).

The physic nut (Jatropha curcas) grows in marginal areas where important crop species are not able to survive (Francis et al., 2005). Besides, it presents high economic potential due to its seed oil quality, which can be converted into biodiesel by the industry (Silva et al., 2009a). Although this species had shown satisfactory yield under constraining conditions of semiarid regions such as drought and high temperature, the plant physiological responses to salinity are not yet well known, especially in terms of key process like the photosynthesis.

This study was designed to evaluate the photosynthesis tolerance of physic nut young plants to salt stress. Changes in leaf gas exchange, photochemistry activity, and salt stress indicators (leaf Na⁺ and Cl⁻ concentrations and K⁺/Na⁺ ratios) were measured after 7 and 14 days of salt stress and 3-d recovery.

**Material and Methods**

The experiment was carried out under greenhouse conditions, localized in the Universidade Federal do Ceará, Fortaleza, Ceará, Brazil (3° 44′ S, 38° 33′ W, at sea level), where the environmental conditions were: minimum and maximum mean air temperature of 24 and 36°C, respectively; mean air relative humidity of 65%; maximum photosynthetic photon flux density (PPFD) of approximately 700 μmol m⁻² s⁻¹. Jatropha curcas L. seeds, genotype FT-2, were previously selected taking into account the seed size and weight. Eight days after germination in sand, seedlings were transferred to plastic pots (2 L), containing Hoagland and Arnon (1950) nutrient solution (pH 6.0) with one-forth strength in the first week and full strength afterwards.

The nutrient solution was supplied with 100 mmol L⁻¹ NaCl and then plants were subjected to this stressful treatment during 14 days. The NaCl was added gradually (50 mmol NaCl L⁻¹ d⁻¹) into solution in order to avoid osmotic shock. The treatment with nutrient solution in absence of NaCl was taken as control. At the end of two weeks of treatment, the salt-stressed plants were returned to control conditions for three days. In a previous experiment, a three-day period was sufficient for the full recovery of physic nut young plants subjected to 50 mmol L⁻¹ NaCl.

Leaf gas exchange was measured with an infrared gas analyzer (LCi, ADC, Hoddesdon, UK), operating in open system and with air flow of 200 mL min⁻¹. Measurements of leaf CO₂ assimilation rate (A), transpiration (E), stomatal conductance (gs) and intercellular CO₂ concentration (Ci) were taken. The instantaneous carboxilation efficiency (A/Ci) was calculated (Zhang et al., 2001).

The chlorophyll fluorescence was evaluated with a modulated fluorimeter (FMS2, Hansatech, King’s Lynn, UK). Minimum (Fo), maximum (Fm) and maximum variable (Fv=Fm-Fo) fluorescence intensities were sampled under steady-state conditions in dark-adapted (30 min) leaves. In addition, measurements were also taken under light-adapted conditions, being referred as Fo’ (minimum) and Fm’ (maximum). The Fo’ signal was measured after PSI excitation by far-red light. The fluorescence signal under light-adapted conditions before the saturation pulse is referred as F ′, and the variable fluorescence signal under light conditions is ΔF=Fm’-F ′.

The following photochemical variables were calculated: maximum (Fv/Fm) and actual (ΔF/Fm’) quantum yield of primary photochemistry. Apparent electron transport rate (ETR= ΔF/Fm’ × PPFD × 0.5 × 0.84), photochemical [qP= (Fm’-Fs)/(Fm’-Fo’)] and non-photochemical [NPQ= (Fm-Fm’)/Fm’] quenching (Rohácek, 2002). For ETR calculation, 0.5 was used as the fraction of excitation energy distributed to PSII and 0.84 as the fraction of the incoming light absorbed by leaves (Schreiber et al., 1998). Fo’ is the basal fluorescence yield measured after PSI excitation by far-red light. The ratio ETR/A was calculated to estimate the use of electrons in other processes not related to the photosynthetic CO₂ assimilation rate (Ribeiro et al., 2009). Therefore, an increase in ETR/A indicates that more electrons are driven to other sinks (e.g., photorespiration, nitrogen metabolism or Mehler reaction), suggesting a stressful condition.

Leaf gas exchange and chlorophyll fluorescence were measured simultaneously, in fully expanded and mature leaves of plants exposed to 25°C and PPFD of 590 μmol m⁻² s⁻¹ supplied by an external light source. Those measurements were taken after 7-d and 14-d of treatment (0 and 100 mmol L⁻¹ NaCl), and repeated again three days after returning plants to the nutrient solution without the presence of NaCl.

At the end of experiment, leaves were sampled, frozen and stored at -80°C. After lyophilization, samples were placed into hermetically closed tubes containing deionized water and heated under water-bath at 100°C for 1 h. The extracts were then filtered and used for determination of Na⁺ and K⁺ contents by flame photometry. The Cl⁻ content was determinate through titration with AgNO₃, as described by Silveira et al. (2009).

The experiment was arranged in a completely randomized design, with two factors: NaCl (0 and 100 mmol L⁻¹); time (seven and 14 days of salt stress and three days of recovery). Data were subjected to analysis of variance and mean from four replicates were compared by the Tukey test at the 0.05 level of confidence.

**Results and Discussion**

Leaves of physic nut young plants treated with NaCl (100 mmol L⁻¹) were affected by the Na⁺, Cl⁻ and K⁺ con-
centrations compared to control plants (Table 1). After seven days of treatment, the Na⁺ and K⁺ concentrations reached values around 603 and 581 mmol (kg DM)⁻¹ respectively, while the K⁺/Na⁺ ratio was reduced from 5.40 to 0.96 in stressed plants. In spite of the Na⁺ accumulation associated with a reduction in K⁺ concentration, and consequent decrease in K⁺/Na⁺ ratio, in the treated plants, the saline condition was not sufficient to induce an ionic stress and a great disturbance in the leaf ion homeostasis, taken as basis the Na⁺ and K⁺ concentrations. In fact, Silva et al. (2009b) observed that toxicity symptoms in physic nut leaves appeared only when the leaf K⁺/Na⁺ ratio is lower than 0.50.

After seven days of treatment with 100 mmol L⁻¹ NaCl, the leaf Cl⁻ content was just 326 mmol (kg DM)⁻¹ (Table 1). This concentration represents only 54% of that noticed for the Na⁺ concentration, evidencing that *J. curcas* is a species more excluder of Cl⁻ than Na⁺. The stress imposed by NaCl was predominantly osmotic over the ionic effects. This evidence is consistent with the results obtained by Silva et al. (2009a) working with several NaCl levels under experimental conditions similar to those employed in this current study. In addition, this species actually has a high affinity for Na⁺ and/or greater ability to exclude Cl⁻ ions from roots and leaves (Silva et al., 2009b).

After 14-d of exposure to salt stress, the Na⁺ and Cl⁻ concentrations reached very high levels, possibly achieving toxic values. The Na⁺ and Cl⁻ concentrations were about 1,721 and 1,498 mmol (kg DM)⁻¹ respectively. These concentrations of Na⁺ and Cl⁻ ions are able to trigger salt toxicity symptoms in physic nut leaves such as leaf chlorosis followed by necrotic areas (Silva et al., 2009b), as shown in the Figure 1. Corroborating these observations, the leaf K⁺ concentration decreased strongly in salt-stressed plants, varying from 1,071 (control) to 423 mmol (kg DM)⁻¹. As a consequence, the K⁺/Na⁺ ratio was reduced from 5.0 to 0.24, evidencing clearly that after 14-d of exposure the salinity induced great disturbances in the K⁺-Na⁺ ionic homeostasis (Munns and Tester, 2008).

The salt-induced osmotic effects after 7-d and the toxic ionic effects after 14-d of salt exposure were evidenced by the appearance of visual symptoms in physic nut leaves (Figure 1). Indeed, after 7-d of treatment the leaves exhibited minor senescence symptoms while the leaves showed drastic toxicity visual symptoms after 14-d as indicated by intense necrotic areas. These symptoms persisted even after the NaCl removal from the nutrient medium (recovery). After 3-d recovery, the Na⁺ and Cl⁻ concentrations in the pre-stressed plants decreased by 15% and 17% only, respectively while that the K⁺/Na⁺ ratio was not changed, in comparison to the 14-d stressed plants (Table 1). Thus, most *J. curcas* plants suffered an osmotic stress after 7-d. However, a strong ionic stress was established in physic nut leaves in the following seven days, i.e., after 14-d of exposure to NaCl.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NaCl</th>
<th>Days after treatment</th>
<th>Recovery</th>
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<tr>
<td></td>
<td>mmol L⁻¹</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>Na⁺</td>
<td>0</td>
<td>133 Bb</td>
<td>215 Ab</td>
</tr>
<tr>
<td>(mmol (kg DM)⁻¹)</td>
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<td>603 Ca</td>
<td>1,721 Aa</td>
</tr>
<tr>
<td>Cl⁻</td>
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<td>102 Bb</td>
<td>147 Ab</td>
</tr>
<tr>
<td>(mmol (kg DM)⁻¹)</td>
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<td>326 Ca</td>
<td>1,498 Aa</td>
</tr>
<tr>
<td>K⁺</td>
<td>0</td>
<td>716 Ba</td>
<td>1,071 Aa</td>
</tr>
<tr>
<td>(mmol (kg DM)⁻¹)</td>
<td>100</td>
<td>581 Ab</td>
<td>423 Cb</td>
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<tr>
<td>K⁺/Na⁺ ratio</td>
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<td>5.4 Aa</td>
<td>5.0 Aa</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.96 Ab</td>
<td>0.24 Bb</td>
</tr>
</tbody>
</table>

Values represented by the same upper case letters, between time of treatment and same lower case letters, into of each time of treatment are different (Tukey, $p < 0.05$).

Figure 1 – Leaf toxicity symptoms in *Jatropha curcas* young plants cultivated in absence and presence of NaCl (100 mmol L⁻¹) during stress period.

Table 1 – Concentrations of Na⁺, Cl⁻ e K⁺ and K⁺/Na⁺ ratio in leaves of physic nut young plants cultivated in absence and presence of NaCl (100 mmol L⁻¹) during 7-d and 14-d and after 3-d of recovery. Values are means of four replicates.
Overall, after the first days of salt exposure glycophyte plants display an osmotic response and that after some weeks the ionic component of the salinity is predominant (Munns and Tester, 2008).

The leaf CO₂ assimilation rate was strongly reduced in plants exposed to salt stress compared to control ones. Plants submitted to NaCl had 23% and 85% reductions after 7 and 14-d of treatment, respectively (Figure 2A). The transpiration rate was reduced in 39% and 84% (Figure 2B), the stomatal conductance by 80% and 97% (Figure 2C) and the intercellular CO₂ concentration by 23% and 47% (data not shown) after 7 and 14-d of treatment, respectively. In contrast, the instantaneous carboxylation efficiency, estimated by the A/Ci ratio (Figure 2D), remained unchanged after 7-d of treatment but it was strongly reduced (73%) after 14-d of exposure to NaCl. Interestingly, after 3-d of recovery the A/Ci ratio did not change as compared to stressed plants (Figure 2D).

The reduction of photosynthesis after seven days of salt treatment was caused only by a stomatal limitation, i.e. reduced CO₂ availability to carboxylation, while both the stomatal and biochemical limitations were observed after 14 days of treatment. As expected, the negative effects caused by salt stress on the leaf gas exchange

Figure 2 – Leaf CO₂ assimilation rate (A), transpiration (B), stomatal conductance (C) and instantaneous carboxilation efficiency (D) in *Jatropha curcas* young plants cultivated in absence and presence of NaCl (100 mmol L⁻¹) during 7-d and 14-d and after 3-d of recovery. White bars represent control plants and gray bars represent stressed plants. Values are means of four replicates. Values represented by the same upper case letters, between time of treatment and same lower case letters, into of each time of treatment are not different (Tukey, p < 0.05).
variables were progressively increased as the time of salt exposure increased. In addition, no improvement in the photosynthesis was observed after the salt removal (recovery treatment), suggesting that after 14-d of treatment irreversible damage on the photosynthesis occurred probably due to ionic toxicity caused in the chloroplasts (López-Climent et al., 2008).

The decrease of photosynthesis associated with low stomatal conductance in plants subjected to saline conditions (Figure 2) is in accordance to Meloni et al. (2003), who observed impairment in the photosynthetic efficiency of cotton cultivars exposed to 50 and 100 mmol L⁻¹ NaCl. On the other hand, decreases in intercellular CO₂ concentration and A/Ci ratio also indicate that salt stress affected the photosynthesis by metabolic limitation. Reductions of A/Ci ratio are probably associated with a decrease of Rubisco carboxylase activity, which occurred in parallel with Na⁺ and Cl⁻ intense accumulation in leaf tissues (Table 1). Thus, the reduction of photosynthesis may, at least in part, be a direct effect of Na⁺ and Cl⁻ ions, as observed in sorghum (Netondo et al., 2004) and orange (López-Climent et al., 2008) plants.

Regarding to photochemistry effects, maximum quantum yield (Fv/Fm) was not affected by salinity, whereas the actual quantum yield of primary photochemistry (ΔF/Fm′) decreased (39%) after 14-d of exposure to NaCl (Figure 3A,B). The photochemical quenching (qP) was not affected by salt stress, while the non-

![Figure 3](image-url)
photochemical quenching (NPQ) increased in plants subjected to salt stress (Figure 3C,D). The increase of NPQ was already significant after seven days of treatment and progressively increased after 14-d of salt stress (Figure 3D).

Interestingly, this photochemical parameter increased in the recovery treatment. The reduction of ΔF/ΔFm’ accompanied by a decrease in the apparent electron transport rate (data not shown) in the stressed plants differs from results reported by Lu et al. (2002), who did not observe effects of salt stress in photochemical reactions of S. salsa leaves. Impairments in photochemistry of plants exposed to salinity may be related to possible damages in primary electron acceptors, such as plastoquinone pool (Foyer and Noctor, 2000). In addition, the continuous increase of NPQ during stress and recovery treatments suggest the activation of a protective mechanism for dissipation of excessive energy not used in photochemical reactions (Ribeiro et al., 2009; Rohácek, 2002). However, this protective defense was not sufficient to avoid oxidative and irreversible damage after 14-d of treatment, as suggested by the toxicity symptoms in leaves (Figure 1). Our data reveal a reasonable photochemical activity even under ionic stress, which was not compatible with the photosynthetic rates (Figures 2 and 3). In fact, full inhibition of PSII photochemistry occurs only under severe oxidative damage (Chagas et al., 2008).

After 14-d under salt stress, plants showed ETR/A ratio 4-fold higher than the control. This difference was maintained even after the recovery period (Figure 4). The increase in ETR/A ratio represents an imbalance between the electron flow and the CO₂ assimilation during photosynthesis, which is frequently associated with increases in oxygenase activity of Rubisco and might represent an electron flow to other physiological processes rather than to CO₂ assimilation reactions (Baker et al., 2007; Ribeiro et al., 2009). Thus, the occurrence of increases in ETR/A and decreases in A/CI might indicate a loss of photosynthetic efficiency in physic nut plants under salinity, especially in the ionic phase of salt stress.

The accumulation of toxic ions (Na⁺ and Cl⁻) accompanied by decreases in K⁺ concentration and severe photosynthetic damages indicate that physic nut young plants are sensitive to high salinity caused by NaCl. The absence of any recovery in photosynthesis, after three days of salt removing, also indicates that high Na⁺ and Cl⁻ leaf concentrations might cause acute damages on the photochemical and gas exchange of the photosynthesis.

Conclusions

Physic nut young plants are sensitive to high salinity, showing high leaf Na⁺ and Cl⁻ concentrations and very low K⁺/Na⁺ ratios, after 14 days of exposure to 100 mmol L⁻¹ NaCl. Under this condition, the salt-induced ionic toxicity effects are capable to induce acute photosynthetic damages (photochemistry and gas exchange) due to stomatal and biochemical limitations.

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