Journal of Plant Physiology 171 (2014) 23–30

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.com/locate/jplph

Physiology

# Dissipation of excess photosynthetic energy contributes to salinity tolerance: A comparative study of salt-tolerant *Ricinus communis* and salt-sensitive *Jatropha curcas*☆

Milton C. Lima Neto, Ana K.M. Lobo, Marcio O. Martins, Adilton V. Fontenele, Joaquim Albenisio G. Silveira*

*Departamento de Bioquímica e Biologia Molecular/INCTsal-CNPq/MCT, Laboratório de Metabolismo de Plantas, Universidade Federal do Ceará, CP 6004, CEP 60455-970 Fortaleza, Ceará, Brazil*

ARTICLE INFO

*Article history:*
Received 25 June 2013
Received in revised form 4 September 2013
Accepted 5 September 2013
Available online 3 October 2013

*Keywords:*
Castor bean
Physic nut
Photoprotection
Photosynthesis
Salt stress

ABSTRACT

The relationships between salt tolerance and photosynthetic mechanisms of excess energy dissipation were assessed using two species that exhibit contrasting responses to salinity, *Ricinus communis* (tolerant) and *Jatropha curcas* (sensitive). The salt tolerance of *R. communis* was indicated by unchanged electrolyte leakage (cellular integrity) and dry weight in leaves, whereas these parameters were greatly affected in *J. curcas*. The leaf $Na^+$ content was similar in both species. Photosynthesis was intensely decreased in both species, but the reduction was more pronounced in *J. curcas*. In this species biochemical limitations in photosynthesis were more prominent, as indicated by increased $C_i$ values and decreased Rubisco activity. Salinity decreased both the $V_{cmax}$ (*in vivo* Rubisco activity) and $J_{max}$ (maximum electron transport rate) more significantly in *J. curcas*. The higher tolerance in *R. communis* was positively associated with higher photorespiratory activity, nitrate assimilation and higher cyclic electron flow. The high activity of these alternative electron sinks in *R. communis* was closely associated with a more efficient photoprotection mechanism. In conclusion, salt tolerance in *R. communis*, compared with *J. curcas*, is related to higher electron partitioning from the photosynthetic electron transport chain to alternative sinks.



## Introduction

Salinity affects $CO_2$ photosynthetic assimilation initially causing decreased stomata conductance, which is induced primarily by the osmotic effects of salt excess in roots, involving ABA transport and resulting in low $CO_2$ supply to Rubisco. In a second phase, salinity might cause biochemical effects on photosynthesis that can restrict both the photochemical and Calvin cycle reactions (Duarte et al., 2013). Impairment in $CO_2$ assimilation induces an imbalance in the overall photosynthetic process, particularly in the presence of high light intensity. Under these conditions, excess electron from the photochemical phase is accumulated in thylakoid membranes, which causes photoinhibition (Parvaiz and Satyawati, 2008). Plants display several strategies to protect the photosystems against photoinhibition and photodamage (Takahashi and Badger, 2011). These protective mechanisms are associated with several reactions at photochemical level (down-regulation in light harvesting, excess energy dissipation by non-photochemical quenching, increase in water-water cycle and cyclic electron flow) and other important biochemical pathways such as the ascorbate-glutathione cycle, photorespiration and, nitrate and ammonia assimilation (Osmond et al., 1997; Niyogi, 1999; Flexas and Medrano, 2002; Takahashi and Murata, 2008). The role of the efficiency of these processes in salt tolerance is possibly species-dependent but the involved mechanisms are actually not understood.

Nitrate assimilation is very important to plant growth, and it is a process very sensitive to salinity (Masclaux-Daubresse et al., 2010). The overall nitrate assimilation pathway is closely linked to photosynthesis because nitrite and ammonia assimilation occur inside chloroplasts and they are strong consumers of electrons from reduced ferredoxin (Foyer and Noctor, 2002). Nitrate and $CO_2$ assimilation are two processes that are coordinately regulated by mechanisms that remain not completely elucidated (Foyer and Noctor, 2002). There is an important open question: which process is most sensitive to salinity: nitrate or $CO_2$ assimilation? Nitrate reduction is catalyzed by nitrate reductase and occurs in the cytosol, producing nitrite that enters into the chloroplasts (Silveira et al., 2001). Nitrate reduction consumes two electrons from NAD(P)H, which might be supplied indirectly by the photosynthetic electron transport.

☆ This article is part of a special issue entitled "Salt stress resistance - multisite regulation in focus".

* Corresponding author. Tel.: +55 8533669821; fax: +55 8533669821.
*E-mail address:* silveira@ufc.br (J.A.G. Silveira).

0176-1617/$ – see front matter © 2013 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.jplph.2013.09.002

Nitrite reduction is catalyzed by nitrite reductase into chloroplasts, consuming six electrons from reduced ferredoxin (Sivasankar and Oaks, 1996). This reaction is a strong alternative sink for the photosynthetic electron transport (PET) chain, recovering oxidized ferredoxin to accept new electrons from PSII and PSI. The ammonia produced is quickly assimilated by the GS/GOGAT cycle, which consumes two electrons from reduced ferredoxin and one ATP molecule per assimilated ammonia (Foyer and Noctor, 2002). Some studies have revealed that this process might mitigate the negative effects caused by salinity in *Jatropha curcas* (Aragão et al., 2012). Moreover, the amino acid metabolism in chloroplasts is extremely active, directly or indirectly consuming photosynthetic electrons as reducing power and ATP (Masclaux-Daubresse et al., 2010). Because of these characteristics, nitrate assimilation is frequently considered a part of the whole photosynthesis ("photosynthetic nitrate assimilation").

Photorespiration ($P_R$) is another important photoprotection mechanism. It originates from the oxygenation of RuBP by Rubisco and leads to the formation of one molecule of 3-phosphoglycerate and one molecule of phosphoglycolate. The photorespiratory cycle allows the conversion of this compound into 3-phosphoglycolate by a series of reactions that involve three different compartments, chloroplasts, peroxisomes and mitochondria, which releases $CO_2$ and $NH_3$ (Bagard et al., 2008). The $NH_3$ released by $P_R$ is re-assimilated *via* GS/GOGAT in the chloroplast consuming reduced ferredoxin and ATP. $P_R$ has the potential to sustain photons in a non-assimilatory pathway, protecting the photosynthetic apparatus against photo-oxidation (Niyogi, 1999) and photoinhibition (Osmond et al., 1997). In addition, photorespiration is very important in biochemical recycling, particularly to nitrogenous compounds under restrictive metabolic conditions such as salinity (Foyer et al., 2009). Additionally, the nitrate assimilation could interact with $P_R$ through the impairment in the nitrate reductase activity by restriction of NADH (Foyer et al., 2009).

*J. curcas* and *Ricinus communis* are species that are well adapted to arid and semiarid regions as potential crops for biofuel production (Achten et al., 2008; Reddy and Matcha, 2010). In these regions, saline soils extensively exist, causing salt stress in plants (Janmohammadi et al., 2012; Rodrigues et al., 2013). Our group has recently demonstrated that *J. curcas* is a salt-sensitive species (Silva et al., 2013). Conversely, some reports have demonstrated that *R.communis* displays some characteristics of salt tolerance such as $Na^+$ exclusion from leaves after long-term exposure to high NaCl concentrations (Sun et al., 2013). Therefore, because these species display contrasting acclimation to salinity, it is important to elucidate the involvement of photosynthesis in salt tolerance. Photorespiration, nitrate assimilation and cyclic electron flow are important alternative sinks for excess photosynthetic electrons. These processes isolate or in combination, could alleviate the adverse effects on photosynthesis caused by salinity (Aragão et al., 2012).

We postulated that alternative sinks to excess electron from the photochemical phase could mitigate the negative salinity effects, contributing to salt tolerance. In the current study, we tested the hypothesis that plants with a higher intensity of nitrate assimilation, photorespiration and electron cyclic flow are more tolerant to salinity. The physiological significance of these processes for salt stress is discussed.

## Material and methods

### *Plant material and growth conditions*

*Jatropha curcas* (L.), cultivar FT2, provided by Fazenda Tamanduá, Brazil and *Ricinus communis* (L.) seeds, cultivar BRS 149 nordestina, provided by EMBRAPA, Brazil, were employed in the current study. The seeds were previously selected by size and weight and were germinated in sand. Fifteen days after germination, seedlings were transferred to plastic pots (4 L) containing half-strength Hoagland and Arnon (1950) nutrient solution at pH 6.0. The nutrient solution was replaced every two weeks. The pH of the solution was measured every two days and adjusted when necessary. Plants were grown under natural conditions in a greenhouse situated in a semiarid region in Fortaleza city, Brazil (3°44′38″ S and 38°34′11″ W, 31 m altitude). The average values of environmental parameters inside the greenhouse over the experimental period were as follows: maximum/minimum temperature, 39 °C/24 °C; relative humidity, 62%; maximum photosynthetic photon flux density (PPFD), 1200 $\mu mol\ m^{-2}\ s^{-1}$; photoperiod, 12 h. The salt stress was imposed in 45-day-old plants. In this period, both species presented a similar phenological stage, characterized by three pairs of fully expanded leaves. The nutrient solution was supplied with 100 mM NaCl, and the plants were subjected to this stressful condition for 6 days, a period when stressed plants showed significant decreases in gas exchange parameters and visual symptoms of salt injury. NaCl was gradually added (50 $mM\ d^{-1}$) to the solution to avoid osmotic shock. Plants cultivated in nutrient solution without NaCl were used as controls.

### *Gas exchange, chlorophyll a fluorescence, photorespiration and P700 absorption measurements*

For evaluation of gas exchange and photochemical responses, plants were transferred to a growth chamber under the controlled conditions of temperature (29° C), VPD (1.2 kPa) and PPFD (700 $\mu mol\ m^{-2}\ s^{-1}$). After one hour of the acclimation period in the growth chamber, the third fully expanded leaf was monitored. The net $CO_2$ assimilation rate ($P_N$), transpiration ($E$), stomatal conductance to water vapor ($g_s$) and intercellular $CO_2$ partial pressure ($C_i$) were measured using a portable infra-red gas analyzer system, equipped with an LED source and a leaf chamber (IRGA LI-6400XT, LI-COR, Lincoln, NE, USA). The $P_N$ was measured in response to changes in the photosynthetic photon flux density (PPFD) and intercellular $CO_2$ partial pressure ($C_i$). Each one of these conditions was separately controlled in the IRGA leaf chamber. For instantaneous measurements, the PPFD was fixed at 1500 $\mu mol\ m^{-2}\ s^{-1}$, 28 °C, VDP between 1.0 and 1.5 kPa, and external $CO_2$ was fixed at atmospheric partial pressure (38 Pa). The $P_N$–PPFD and $P_N$–$C_i$ fitting curves were determined according to the models proposed Lieth and Reynolds (1987) and Sharkey et al. (2007), respectively. The following parameters associated with photosynthetic efficiency were determined: the maximum photosynthetic rate ($P_{Nmax}$), maximum Rubisco carboxylation rate ($V_{cmax}$), maximum rate of photosynthetic electron transport ($J_{max}$) and respiration in the dark ($R_N$).

*In vivo* chlorophyll *a* fluorescence was measured using a LI-6400-40 Leaf Chamber Fluorometer (LI-COR, Lincoln, NE, USA) coupled with the IRGA. The actinic light utilized for measuring the gas exchange and chlorophyll *a* fluorescence was 1500 $\mu mol\ m^{-2}\ s^{-1}$ PPFD. The fluorescence parameters were measured using the saturation pulse method (Schreiber et al., 1994) in leaves exposed to light and 30-min dark-adapted conditions. The intensity and duration of the saturation light pulse were 8000 $\mu mol\ m^{-2}\ s^{-1}$ and 0.7 s, respectively. The amount of blue light was set to be 10% of the PPFD to maximize stomatal aperture (Flexas et al., 2007). The following parameters were assessed: the maximum quantum yield of PSII [Fv/Fm = (Fm – Fo)/Fm] was measured under 30-min dark-adapted conditions, the effective quantum yield of PSII [ΔF/Fm' = (Fm – Fs)/Fm'] was measured in leaves exposed to actinic light of 1500 $\mu mol\ m^{-2}\ s^{-1}$ PPFD. The photochemical quenching coefficient was calculated as qP = (Fm' – Fs)/(Fm' – Fo'),

the non-photochemical quenching coefficient was calculated as NPQ = (Fm – Fm')/Fm' and the actual flux of photons from the PSII was calculated as ETR = (ΔF/Fm' × PPFD × 0.5 × 0.84). To evaluate the ETR, 0.5 was used as the fraction of excitation energy distributed to PSII, and 0.84 was used as the fraction of incoming light absorbed by the leaves. The Fm and Fo parameters correspond to maximum and minimum fluorescence of dark-adapted leaves, respectively; Fm' and Fs are the maximum and steady-state fluorescence in the light-adapted leaves, respectively, and Fo' is the minimum fluorescence after the far-red illumination of the previously light-exposed leaves (Maxwell and Johnson, 2000).

The photorespiration rate ($P_R$) was estimated as described by Bagard et al. (2008) from the measurements of gas exchange ($P_N$ and $R_d$) and chlorophyll *a* fluorescence-derived ETR parameters using the following equation: $P_R = 1/12[\text{ETR-4}(A + R_d)]$. The redox state of the PSI primary donor, P700, was measured using a Walz DUAL-PAM 100 (Walz, Effeltrich, Germany). The following parameters were assessed: the photochemical quantum yield of PSI [Y(I)], the estimated electron transport rate of PSI (ETRI), the non-photochemical quantum yield of PSI [Y(ND)], donor-side limitation of PSI and [Y(NA)] a the non-photochemical acceptor site limitation of PSI (Yamori et al., 2011). The relative cyclic electron flow (CEF) was estimated by the ETRI/ETRII ratios according to Yamori et al. (2011).

### *Water relations, electrolyte leakage and $Na^+$ and $K^+$ determinations*

The leaf relative water content (RWC) was calculated as follows: RWC = [(FW – DW)/(TW – DW)] × 100, where FW is the fresh weight, TW is the turgid weight measured after 6 h of saturation in deionized water at 4 °C in the dark and DW is the dry weight determined after 48 h in an oven at 75 °C (Silveira et al., 2009). The leaf pre-dawn water potential (Ψw) was evaluated immediately after sampling (3:30 am) using the pressure chamber method (Scholander et al., 1965). Electrolyte leakage (membrane damage) or cellular viability was measured as described previously by Silva et al. (2010). Twenty leaf discs (1.0 cm in diameter) were placed in test tubes containing 20 mL of deionized water. Flasks were incubated in a shaking water bath at 25 °C for 12 h, and the electric conductivity in the medium (L1) was measured. Next, the discs were boiled at 95 °C for 60 min and cooled to 25 °C, and the electric conductivity (L2) was measured again. The relative electrolyte leakage (EL) was estimated using the following equation: EL = L1/L2 × 100. The content of $Na^+$ and $K^+$ ions was determined by flame photometry (Ferreira-Silva et al., 2008).

### *Determination of nitrate, ammonia, proline and glycinei betaine*

The leaf nitrate and ammonium were extracted with hot water (100 °C) and were determined using the methods of Cataldo et al. (1975) and Weatherburn (1967), respectively. The proline concentration in leaves was determined according to the method of Bates et al. (1973) by the formation of the complex proline-ninhydrin measured at 520 nm *via* a spectrophotometer. The glycine betaine (GB) concentrations in the leaves were determined according to Grieve and Grattan (1983) by the formation of periodide crystals dissolved in 1,2-dichloroethane. The absorbance was measured at 365 nm.

### *Nitrate reductase and glutamine-synthetase activities*

The nitrate reductase (NR, EC 1.6.6.1) activity was measured using an *in vivo* method according to Hageman and Hucklesby (1971), described in detail by Silveira et al. (2001). The reaction mixture contained a buffer (100 mM potassium phosphate, pH 7.5), 1 mM NADH, and 250 mM $KNO_3$. The assay was initiated by adding leaf extract and was conducted at 30 °C for 15 min. The reaction was stopped by the addition of 2 mL of (1:1) 1% sulfanilamide in 2.4 M HCl:0.02% (m/v) N-1-naphthyl-ethylenediamine. The $A_{540}$ of the supernatant was measured. NR activity was expressed as μmol $NO_2$ $g^{-1}$ MF $h^{-1}$.

For glutamine synthetase (GS, EC 6.3.1.2) extraction, the medium contained 100 mM potassium phosphate buffer (pH 7.4) and 1 mM EDTA. GS activity was determined using the hydroxamate biosynthetic method as described by Berteli et al. (1995). The reaction mixture consisted of 100 mM Tris–HCl buffer (pH 7.0), 3.5 mM ATP (pH 7.0), 60 mM $MgSO_4$, 20 mM hydroxylamine hydrochloride neutralized with HCl and 50 mM Na-glutamate. The reaction was started by the addition of enzyme extract, and the mixture was incubated at 30 °C for 30 min. A control was performed omitting hydroxylamine from the reaction mixture. A standard curve was constructed with γ-glutamyl hydroxamate. GS activity was expressed as μmol γ-glutamyl hydroxamate (GGH) $g^{-1}$ MF $h^{-1}$.

### *Preparation of crude extracts and determination of enzyme activities*

To prepare the crude extracts, fresh leaf samples were ground to a fine powder in liquid $N_2$ with a mortar and pestle and extracted with ice-cold 100 mM Tris–HCl buffer (pH 8), containing 0.1 mM EDTA, 1 mM ascorbic acid, 20% glycerol, 3% PEG 6000 and 30 mM DTT. The enzymatic extract was stored at −20 °C until the determinations of the activities, which were performed immediately. CAT (EC 1.11.1.6) activity in the crude extract was measured following the oxidation of $H_2O_2$ at 240 nm over 300 s in the presence of 50 mM potassium phosphate buffer (pH 7.0) containing 20 mM $H_2O_2$ at 30 °C (Havir and McHalen, 1987). The CAT activity was determined according to the molar extinction coefficient of $H_2O_2$ (36 $M^{-1}cm^{-1}$) and was expressed as μmol $H_2O_2$ $mg^{-1}$ protein $min^{-1}$. GO (EC 1.1.3.15) activity was assayed by the formation of a glyoxylate-phenylhydrazone complex at 324 nm (Baker and Tolbert, 1966). The GO assay mixture contained 100 mM of phosphate buffer (pH 8.3), 40 mM glycolic acid, 100 mM L-cysteine and 100 mM phenylhydrazine. The reaction was started with the addition of the 1 mM FMN, and the absorbance was monitored over 300 s. GO activity was calculated using the molar extinction coefficient of the glyoxylate-phenylhydrazone complex (17 $mM^{-1}$ $cm^{-1}$) and was expressed as μmol glyoxylate $mg^{-1}$ protein $min^{-1}$. Rubisco activity was measured spectrophotometrically from the rate of NADH oxidation at 340 nm (Reid et al., 1997). For the determination of the initial Rubisco activity, the extract was added to 900 μL of the assay mixture, and the reaction was initiated with the addition of 0.5 mM RuBP. For measurement of total activity, the reaction was started after 15 min of incubation of the mixture reaction in the absence of RuDP. Thereafter, 0.5 mM RuBP was added, and the total activity was measured from NADH oxidation at 340 nm. Both the initial and total Rubisco activities were expressed as μmol $CO_2$ $m^{-2}$ $s^{-1}$. The Rubisco activation state was calculated using the ratio initial activity/total activity and was expressed as a percentage (%).

### *Statistical analysis and experimental design*

The experiments were arranged in a completely randomized design in a 2 × 2 factorial (two plant species × two NaCl levels) and four replicates, each consisting of an individual plant in a pot. Data

**Table 1**
Leaf dry matter (DM), relative water content (RWC), water potential (Ψw) and membrane damage in leaves of *Ricinus communis* and *Jatropha curcas* plants subjected to 6 days of 100 mM NaCl (salt) or control treatment. Upper-case letters represent different means between treatments for the same species, and lower-case letters represent different means between species for the same treatment (Tukey's test; $P < 0.05$).

| | *R. communis* | | *J. curcas* | |
|---|---|---|---|---|
| | Control | Salt | Control | Salt |
| Leaf DM (g plant$^{-1}$) | 3.64Aa | 3.15Aa | 2.63Ab | 1.62Bb |
| RWC (%) | 79Aa | 66Ba | 70Ab | 65Ba |
| Ψw (MPa) | −0.42Ba | −1.24Ab | −0.22Bb | −1.49Aa |
| Membrane damage (%) | 31Aa | 34Ab | 29Ba | 53Aa |

**Table 2**
$Na^+$, $K^+$, proline and glycine betaine contents in leaves of *Ricinus communis* and *Jatropha curcas* plants subjected to 6 days of 100 mM NaCl (salt) or control treatment. Upper-case letters represent different means between treatments for the same species, and lower-case letters represent different means between species for the same treatment (Tukey's test; $P < 0.05$).

| | *R. communis* | | *J. curcas* | |
|---|---|---|---|---|
| | Control | Salt | Control | Salt |
| $Na^+$ (mmol kg$^{-1}$ DM) | 76Bb | 382Aa | 90Ba | 410Aa |
| $K^+$ (mmol kg$^{-1}$ DM) | 250Ab | 143Bb | 420Aa | 284Ba |
| Proline (μmol g$^{-1}$ DM) | 0.43Bb | 0.64Aa | 0.24Bb | 0.43Aa |
| Glycine betaine (μmol g$^{-1}$ DM) | 50.56Bb | 180.47Ab | 200.67Aa | 250.76Aa |

were analyzed using ANOVA, and the means were compared using Tukey's test at a confidence level of 0.05.

## Results

### *Growth, water relations and organic and solutes in J. curcas and R. communis*

In the present study, *R. communis* and *J. curcas* young plants were exposed to salt stress (100 mM NaCl) for six consecutive days. Salinity promoted significant reduction in leaf dry matter in *J. curcas* but no change occurred in *R. communis*, compared with the respective controls (Table 1). The leaf relative water content and water potential decreased in both species under salt stress. In addition, salt stress increased the membrane damage (indicated by electrolyte leakage) in *J. curcas* plants (by 83%), whereas this parameter did not change in *R. communis* (Table 1).

Concerning to the inorganic and organic solute contents, salt treatment similarly increased the $Na^+$ content in *R. communis* and *J. curcas* (Table 2). As expected, the leaf $K^+$ content decreased in both species under salinity but the former species exhibited lower $K^+$ concentration. The proline content increased in *R. communis* and *J. curcas* exposed to salinity by 50% and 100%, respectively, but the absolute contents in both species were very low. The glycine betaine content increased by 250% and 25% in *R. communis* and *J. curcas*, respectively, but the latter species presented a content four-fold higher compared with *R. communis* (Table 2).

### *Photochemical, gas exchange parameters and estimated photorespiration in J. curcas and R. communis*

All leaf gas exchange parameters evaluated in this study were affected by the imposed salt stress in both species (Fig. 1). The net photosynthesis ($P_N$) decreased by 82% and 88% in *R. communis* and *J. curcas*, respectively, and transpiration ($E$) and stomatal conductance ($g_s$) decreased by a similar magnitude. Interestingly, the intercellular $CO_2$ partial pressure ($C_i$) decreased in *R. communis* plants exposed to salinity by 25% and, in opposition, increased by 52% in *J. curcas* (Fig. 1D).

Regarding chlorophyll *a* fluorescence parameters, the effective quantum yield of PSII (ΔF/Fm') and photochemical quenching (qP) were similar and strongly reduced in both species exposed to salinity (Fig. 2). The potential quantum yield of PSII (Fv/Fm) decreased slightly in *R. communis* plants (Fig. 2B). Non-photochemical

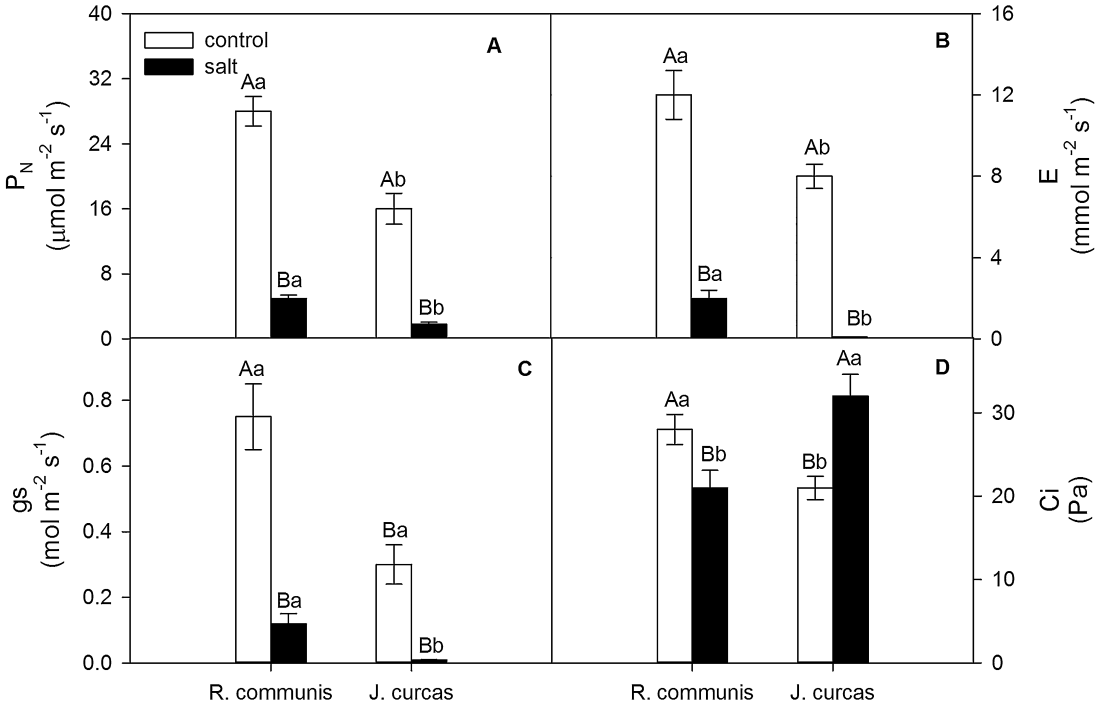


**Fig. 1.** Net photosynthesis (A), transpiration (B), stomatal conductance (C) and $CO_2$ intercellular partial pressure (D) in *R. communis* and *J. curcas* seedlings subjected to 6 days of salinity (100 mM NaCl) or control treatment. Upper-case letters represent significantly different means between treatments within the same species, and lower-case letters represent significantly different means between species and with the same treatment (Tukey's test; $P < 0.05$).

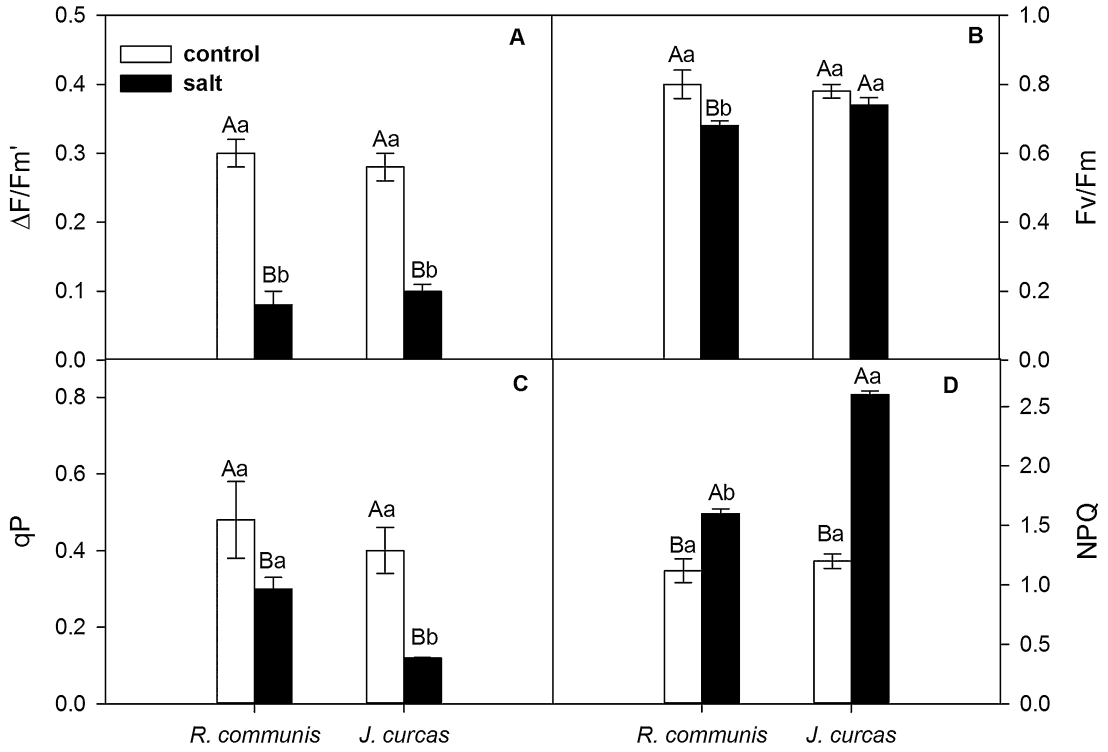


**Fig. 2.** Effective quantum yield of PSII (A), potential quantum yield of PSII (B), photochemical quenching (C) and $CO_2$ intercellular partial pressure (D) in *R. communis* and *J. curcas* seedlings subjected to 6 days of salinity (100 mM NaCl) or control treatment. Upper-case letters represent significantly different means between treatments within the same species, and lower-case letters represent significantly different means between species and with the same treatment (Tukey's test; $P < 0.05$).

quenching (NPQ) was slightly increased by salt stress in *R. communis* and strongly in *J. curcas* (Fig. 2D).

The photosynthetic efficiency parameters estimated from the modeling of $P_N/C_i$ and $P_N$/PPFD curves were significantly affected by salt treatment in both species (Table 3). The maximum photosynthetic rate ($P_{Nmax}$) decreased similarly in both species in response to salinity. The maximum carboxylation rate of Rubisco ($V_{cmax}$) decreased more prominently in *J. curcas*, whereas the maximum

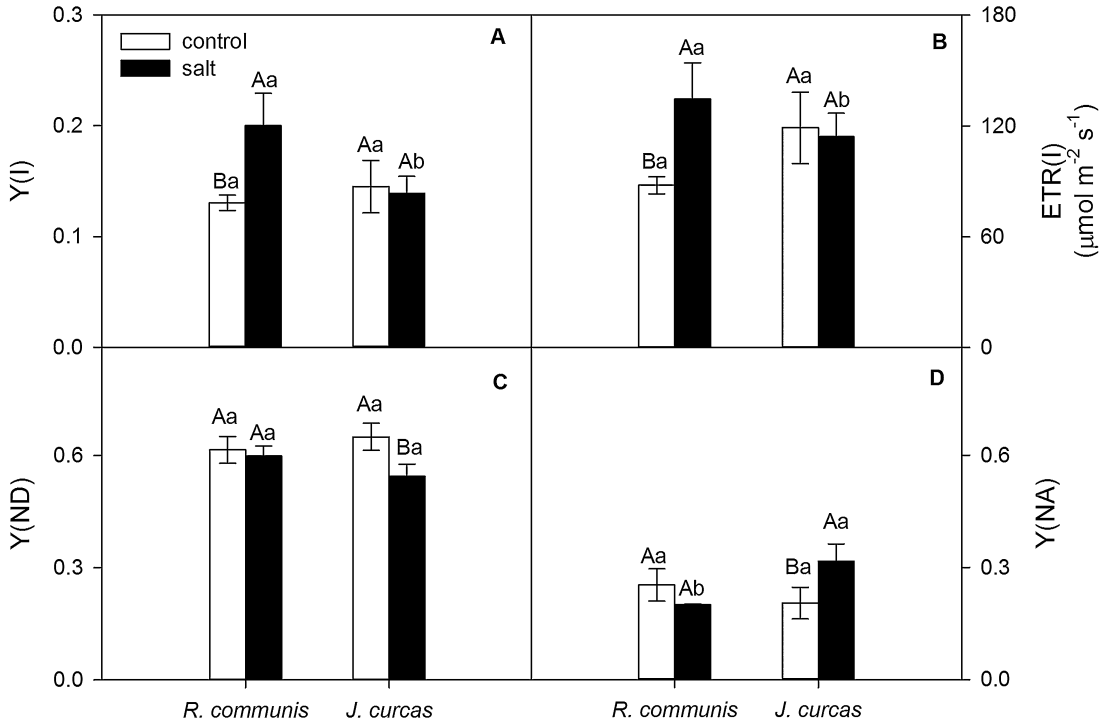


**Fig. 3.** Effective quantum yield of PSI (A), electron transport rate at PSI (B), non-photochemical donor site limitation of PSI (C) and non-photochemical acceptor site limitation of PSI (D) in *R. communis* and *J. curcas* seedlings subjected to 6 days of salinity (100 mM NaCl) or control treatment. Upper-case letters represent significantly different means between treatments within the same species, and lower-case letters represent significantly different means between species and with the same treatment (Tukey's test; $P < 0.05$).

**Table 3**
Parameters calculated from the $P_N$–PPFD and $P_N$–$C_i$ response curves. $P_{Nmax}$ is the maximum $CO_2$ assimilation rate; $R_d$ is respiration in the light; $R_N$ is respiration in the dark; $V_{cmax}$ is the maximum carboxylation rate of Rubisco; $J_{max}$ is the maximum photosynthetic electron transport rate; and *CEF* is the relative cyclic electron flux in *R. communis* and *J. curcas* seedlings subjected to 6 days of 100 mM NaCl (salt) or control treatment. Upper-case letters represent different means among treatments for the same species, and lower-case letters represent different means among species for the same treatment (Tukey's test; $P < 0.05$).

| | *R. communis* | | *J. curcas* | |
|---|---|---|---|---|
| | Control | Salt | Control | Salt |
| $P_{Nmax}$ ($\mu$mol m$^{-2}$ s$^{-1}$) | 29.04Aa | 8.63Ba | 21.22Ab | 6.01Ba |
| $R_N$ ($\mu$mol m$^{-2}$ s$^{-1}$) | 1.78Ba | 2.43Aa | 1.63Aa | 1.94Ab |
| $V_{cmax}$ ($\mu$mol m$^{-2}$ s$^{-1}$) | 124Aa | 86Bb | 111Ab | 28Ba |
| $J_{max}$ ($\mu$mol m$^{-2}$ s$^{-1}$) | 132Aa | 52Ba | 92Ab | 32Bb |
| $P_R$ ($\mu$mol m$^{-2}$ s$^{-1}$) | 3.53Ba | 9.52Ab | 4.32Aa | 5.21Ba |
| *CEF* (r.u.) | 0.48Bb | 2.32Aa | 0.76Ba | 1.85Ab |

electron transport rate ($J_{max}$) decreased similarly in both species. Respiration in the dark ($R_N$) increased in *R. communis* but did not change in *J. curcas*. The estimated photorespiration rate increased greatly in *R. communis* (by two-fold) and slightly in *J. curcas*. The CEF was increased in both species under salt stress but *R. communis* displayed higher values than *J. curcas* under salinity (Table 3).

The effective quantum yield of PSI [Y(I)] and the apparent electron transport at PSI (ETR I) showed the same pattern in both species under salt stress (Fig. 3). Y(I) and ETR I increased in *R. communis* but did not change in *J. curcas* plants (Fig. 3A and B). The non-photochemical donor site limitation of PSI [Y(ND)] was not affected in *R. communis* but was decreased in *J. curcas*. However, the non-photochemical acceptor site limitation of PSI [Y(NA)] was slightly decreased in *R. communis* and, in contrast, was slightly increased in *J. curcas* (Fig. 3C and D).

Salt stress significantly decreased the initial activity of Rubisco in *J. curcas*, but no change was noted in *R. communis* (Fig. 4A). Salinity did not affect the activation state of Rubisco in both studied species (Fig. 4B). GO activity increased by three-fold and by 71% in *R. communis* and *J. curcas*, respectively (Fig. 5B). CAT activity increased in both species under salinity but exhibited values that were almost two-fold higher in *R. communis* than in *J. curcas* (Fig. 5A).

### *Nitrate and ammonia assimilation and nitrate and ammonium concentration*

Salt stress increased the leaf nitrate content in *J. curcas* plants but did not change the leaf nitrate content in *R. communis* (Fig. 6A). The leaf ammonium content was increased in *R. communis* but was decreased in *J. curcas* (Fig. 6B). Leaf NR activity increased by 52% in *R. communis* but was decreased by 67% in *J. curcas* (Fig. 6C). GS activity did not change in both species under salinity, but *R. communis* presented activity that was approximately five-fold higher than that observed for *J. curcas* (Fig. 6D).

## Discussion

In the current study, we demonstrated that *R. communis* young plants are less sensitive to salt stress than *J. curcas*. The better salt acclimation exhibited by the former species is clearly demonstrated by the absence of negative effects caused by salt on leaf growth (dry weight) and the maintenance of cellular integrity, as indicated by the leaf electrolyte leakage. These stress parameters are good indicators of salt acclimation/tolerance in glycophytes. Those favorable indicators were associated with physiological mechanisms that supported better growth in *R. communis* under salinity such as higher $CO_2$ assimilation and nitrate reductase activity in leaves. In addition, *R. communis* displayed lower decreases in Rubisco activity,

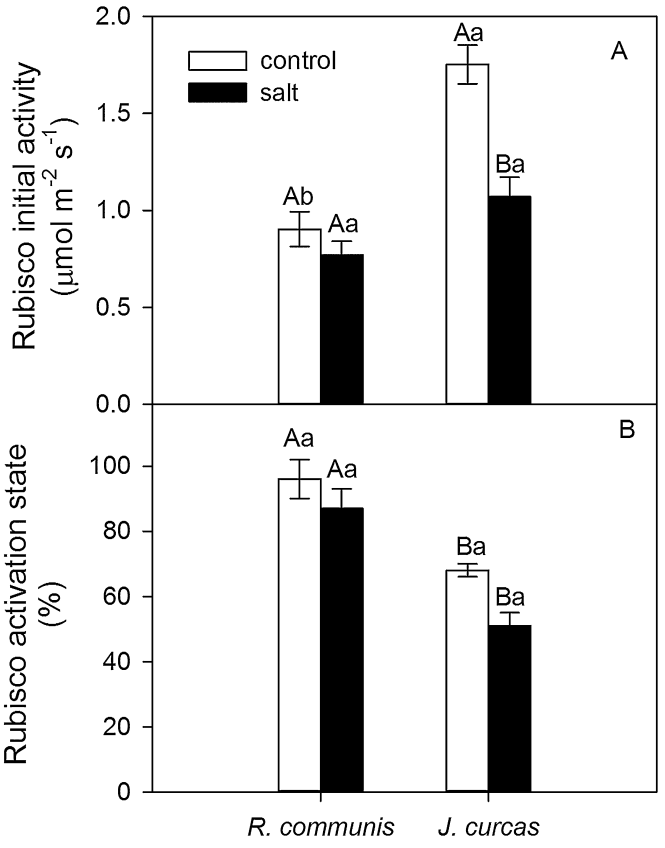


**Fig. 4.** Changes in initial Rubisco activity (A) and Rubisco activation state (B) in *Ricinus communis* and *Jatropha curcas* seedlings subjected to 6 days of 100 mM NaCl (salt) or control treatment. Upper-case letters represent significantly different means between treatments within the same species, and lower-case letters represent significantly different means between species and with the same treatment (Tukey's test; $P < 0.05$).

demonstrating a lower metabolic limitation of photosynthesis than *J. curcas*. Under salt stress *R. communis* exhibited higher activity of PSII (photochemical quenching), compared with *J. curcas* and increased effective quantum yield and electron transport rate in PSI, compared with control. In addition, the estimated CEF was higher in *R. communis* plants exposed to salt compared with *J. curcas*. These parameters associated with the CEF, which is an important mechanism for excess energy dissipation under conditions of impairment of $CO_2$ assimilation (Johnson, 2011) caused by salinity.

Interestingly, the salt-sensitive species *J. curcas*, also exhibited some physiological characteristics favorable to salt acclimation compared with *R. communis*. This species presented higher K/Na ratios and higher $K^+$ concentration in leaves under salinity. In most glycophytes, this first parameter is very important for salt tolerance (Munns and Tester, 2008). However, in the current study, the $Na^+$ content in leaves did not reach high and toxic levels in both species (Silva et al., 2011). It was previously demonstrated that *J. curcas* accumulates high concentrations of $Na^+$ in leaves in presence of 100 mM NaCl after only two weeks (Silva et al., 2011). These results are in agreement with the obtained data in this current study, evidencing that after six days of salt exposure both *J. curcas* and *R. communis* seedlings did not reach toxic $Na^+$ levels in their leaves.

Previous studies with *J. curcas* young plants have suggested that this species is salt sensitive and suffers irreversible damage on the photosynthetic apparatus after a two-week exposure to 100 mM NaCl. *J. curcas* can exhibit a leaf $Na^+$-exclusion mechanism only during approximately one week when the osmotic phase is predominant (Silva et al., 2011). Conversely, some studies have

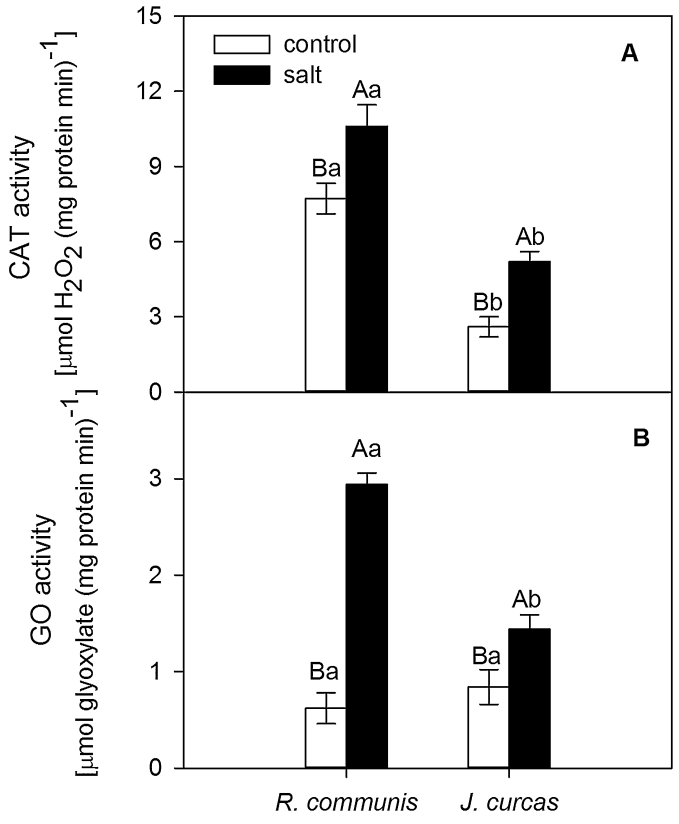


**Fig. 5.** Catalase activity (A) and glycolate oxidase activity (B) in *Ricinus communis* and *Jatropha* curcas seedlings subjected to 6 days of 100 mM NaCl (salt) or control treatment. Upper-case letters represent significantly different means between treatments within the same species, and lower-case letters represent significantly different means between species and with the same treatment (Tukey's test; $P < 0.05$).

demonstrated that *R. communis* is a salt-tolerant species (Pinheiro et al., 2008; Li et al., 2010; Sun et al., 2013). Because both species in this present study exhibited similar leaf-$Na^+$ concentrations, and *R. communis* presented lower K/Na ratios, one central question can be raised here: which others physiological characteristic(s) would have contributed to salt tolerance in *R. communis*? Under salinity, this species exhibited a great stimulation in photorespiration. This situation is particularly important under high salinity when $CO_2$ assimilation is impaired and electron excess is accumulated in thylakoid. In this study the higher photorespiratory rate in *R. communis* was clearly demonstrated by the gas exchange parameter and biochemical indicators (activities of GO, GS and CAT).

The nitrite and ammonia assimilation in photosynthesizing leaves contribute as a strong alternative sink for the (PET) chain, converting the reduced ferredoxin in its oxidized form that can accept new electrons from PSII and PSI. In the present study, the increase in the NR activity in *R. communis* leaves under salinity, associated with maintenance of high GS activity, strongly suggest that nitrate and ammonia assimilation might have contributed to mitigate the negative effects caused by salinity on the photosynthesis.

The salt tolerant species *R. communis* exhibited intriguing results and to some extent, conflicting with literature in terms of nitrate reductase activity and photosynthetic $CO_2$ assimilation. In general, these two processes act coordinately in most species under normal conditions. NaCl stimulated nitrate reductase activity but the photosynthesis was strongly impaired in *R. communis* leaves. Some plant species like soybean has a constitutive form of NR that is more independent on nitrate transport from roots to leaves affected by transpiration than $NO_3^-$-inducible forms (Silveira et al., 2001). Possibly, in salt-treated *R. communis* the levels of NR protein, $NO_3^-$ and cytosolic NADH were favorable to stimulate nitrate reductase activity but not for Rubisco expression and activity. This question is interesting and further studies are needed for elucidation. In addition to stomatal limitation, *J. curcas* also presented biochemical limitations in photosynthesis, as shown by

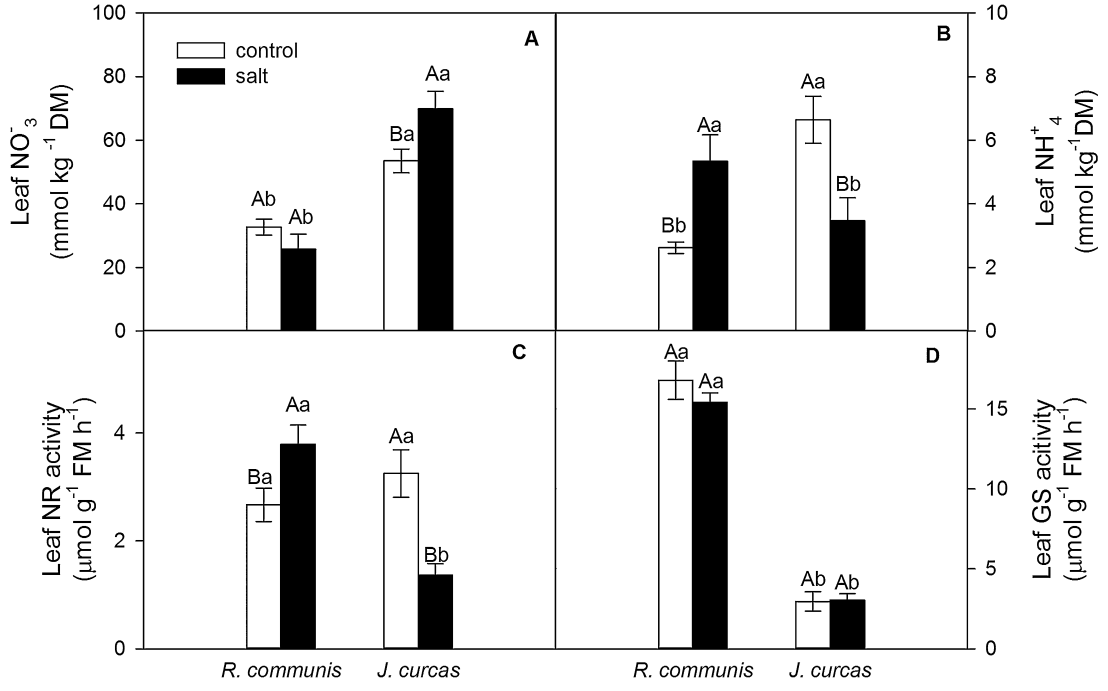


**Fig. 6.** Changes in leaf (A) $NO_3^-$, (B) $NH_4^+$content, (C) nitrate reductase and (D) glutamine synthase activities in *Ricinus communis* and *Jatropha curcas* seedlings subjected to 6 days of 100 mM NaCl (salt) or control treatment. Upper-case letters represent significantly different means between treatments within the same species, and lower-case letters represent significantly different means between species and with the same treatment (Tukey's test; $P < 0.05$).

the increases in $C_i$ values, decrease in Rubisco activity *in vitro* and greater reduction in $V_{cmax}$ and $J_{max}$ compared with *R. communis* plants exposed to salt stress. Conversely, *J. curcas* plants presented an efficient energy excess dissipation mechanism as heat by NPQ under salt stress possibly protecting the PSII against photodamage and photoinhibition. The increase in NPQ is an important mechanism for photoprotection in plants, dissipating the excess energy as heat, avoiding damage at the PSII core (Takahashi and Badger, 2011).

In this study, we reported a non-classical mechanism involved with salt tolerance based on three processes related with excess energy dissipation from the photosynthetic electron transport chain. The salt tolerance of *R. communis* compared with *J. curcas* is associated with higher photorespiration, nitrate assimilation and cyclic electron flow. These processes might contribute to protection against photodamage, photoinhibition and photo-oxidative stress under salinity.

## Acknowledgments

We are grateful to Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP) (Project 2155/Programa Núcleos de Excelência, PRONEX) and INCTsal-CNPq/MCT for financial support.

## References

Achten W, Verchot L, Franken Y, Mathijs E, Singh V, Aerts R, et al. *Jatropha* bio-diesel production and use. Biomass Bioenergy 2008;32:1063–84.

Aragão RM, Silva EM, Vieira CF, Silveira JAG. High supply of $NO_3^-$ mitigates salinity effects through an enhancement in the efficiency of photosystem II and $CO_2$ assimilation in *Jatropha curcas* plants. Acta Physiol Plant 2012;34:2135–43.

Bagard M, Thiec DL, Delacote E, Hasenfratz-Sauder MP, Banvoy J, Gérard J, et al. Ozone-induced changes in photosynthesis and photorespiration of hybrid poplar in relation to the developmental stage of the leaves. Physiol Plant 2008;134:559–74.

Baker AL, Tolbert NE. Glycolate oxidase (ferredoxin-containing form). Meth Enzymol 1966;9:339–40.

Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant Soil 1973;39:205–7.

Berteli F, Corrales E, Guerrero C, Ariza MJ, Pliego F, Valpuesta V. Salt stress increases ferredoxin-dependent glutamate synthase activity and protein level in the leaves of tomato. Physiol Plant 1995;93:259–64.

Cataldo DA, Haroon M, Schrader LE, Yougs VL. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Commun Soil Sci Plant Anal 1975;6:71–80.

Duarte B, Santos D, Marques JC, Caçador I. Ecophysiological adaptations of two halophytes to salt stress: photosynthesis, PS II photochemistry and anti-oxidant feedback – implications for resilience in climate change. Plant Physiol Biochem 2013;67:178–88.

Ferreira-Silva SL, Silveira JAG, Voigt EL, Soares LSP, Viégas RA. Changes in physiological indicators associated with salt tolerance in two contrasting cashew rootstocks. Braz J Plant Physiol 2008;20:51–9.

Flexas J, Medrano H. Energy dissipation in $C_3$ plants under drought. Funct Plant Biol 2002;29:1209–15.

Flexas J, Ribas-Carbó M, Diaz-Espejo A, Galmés J, Medrano H. Mesophyll conductance to $CO_2$: current knowledge and future prospects. Plant Cell Environ 2007;31:602–21.

Foyer CH, Noctor G. Photosynthetic nitrogen assimilation and associated carbon and respiratory metabolism. Dordrecht: Kluwer Academic Publisher; 2002.

Foyer CH, Bloom AJ, Queval G, Noctor G. Photorespiratory metabolism: genes, mutants, energetics, and redox signaling. Annu Rev Plant Biol 2009;60:455–84.

Grieve CM, Grattan SR. Rapid assay for determination of water-soluble quaternary ammonium-compounds. Plant Soil 1983;70:303–7.

Hageman RH, Hucklesby DP. Nitrate reduction from higher plants. Meth Enzymol 1971;23:491–503.

Havir EA, McHalen A. Biochemical and developmental characterization of multiple forms of catalase in tobacco-leaves. Plant Physiol 1987;84:450–5.

Hoagland DR, Arnon DI. The water culture method for growing plants without soil. California Agric Exp Cir 1950:347.

Janmohammadi M, Abbasi A, Sabaghnia N. Influence of NaCl treatments on growth and biochemical parameters of castor bean (*Ricinus communis* L.). Acta Agric Slov 2012;99:31–40.

Johnson GN. Reprint of: physiology of PSI cyclic electron transport in higher plants. Biochim Biophys Acta 2011;1807:906–11.

Li G, Wan S, Zhou J, Yang Z, Qin P. Leaf chlorophyll fluorescence, hyperspectral reflectance, pigments content, malondialdehyde and proline accumulation responses of castor bean (*Ricinus communis* L.) seedlings to salt stress levels. Ind Crops Prod 2010;31:13–9.

Lieth JH, Reynolds JF. The nonrectangular hyperbola as a photosynthetic light response model: geometrical interpretation and estimation of the parameter $\theta$. Photosynthetica 1987;101:363–6.

Masclaux-Daubresse C, Daniel-Vedele F, Dechorgnat J, Chardon F, Gaufichon L, Suzuki A. Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. Ann Bot 2010;105:1141–57.

Maxwell K, Johnson GN. Chlorophyll fluorescence—a practical guide. J Exp Bot 2000;51:659–68.

Munns R, Tester M. Mechanisms of salinity tolerance. Annu Rev Plant Biol 2008;59:651–81.

Niyogi KK. Photoprotection revisited: genetic and molecular approaches. Annu Rev Plant Physiol Plant Mol Biol 1999;50:333–59.

Osmond B, Badger M, Maxwell K, Bjorkman O, Leegood R. Too many photons: photorespiration, photoinhibition and photooxidation. Trends Plant Sci 1997;2:119–21.

Parvaiz A, Satyawati S. Salt stress and phyto-biochemical responses of plants – a review. Plant Soil Environ 2008;54:89–99.

Pinheiro HA, Silva JV, Endres L, Ferreira VM, Câmara CA, Cabral FF, et al. Leaf gas exchange, chloroplastic pigments and dry matter accumulation in castor bean (*Ricinus communis* L) seedlings subjected to salt stress conditions. Ind Crops Prod 2008;27:385–92.

Reddy KR, Matcha SK. Quantifying nitrogen effects on castor bean (*Ricinus communis* L.) development, growth, and photosynthesis. Ind Crops Prod 2010;31:185–91.

Reid CD, Tissue DT, Fiscus EL, Strain BR. Comparison of spectrophotometric and radioisotopic methods for the assay of Rubisco in ozone-treated plants. Physiol Plant 1997;101:398–404.

Rodrigues CRF, Silva EN, Ferreira-Silva SL, Voigt EL, Viégas RA, Silveira JAG. High $K^+$ supply avoids $Na^+$ toxicity and improves photosynthesis by allowing favorable $K^+$:$Na^+$ ratios through the inhibition of $Na^+$ uptake and transport to the shoots of *Jatropha curcas* plants. J Plant Nutr Soil Sci 2013;176:157–64.

Scholander PF, Bradstreet ED, Hemmingsen EA, Hammel HT. Sap pressure in vascular plants: negative hydrostatic pressure can be measured in plants. Science 1965;148:339–46.

Schreiber U, Bilger W, Neubauer C. Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of *in vivo* photosynthesis. In: Schulze ED, Caldwell MM, editors. Ecophysiology of photosynthesis. Berlin: Springer; 1994. p. 49–70.

Sharkey TD, Bernacchi CJ, Farquhar GD, Singsaas EL. Fitting photosynthetic carbon dioxide response curves for C3 plants. Plant Cell Environ 2007;30:1035–40.

Silva EN, Ribeiro RV, Ferreira-Silva SL, Viégas RA, Silveira JAG. Comparative effects of salinity and water stress on photosynthesis, water relations and growth of *Jatropha curcas* plants. J Arid Environ 2010;74:1130–7.

Silva EN, Ribeiro RV, Ferreira-Silva SL, Viégas RA, Silveira JAG. Salt stress induced damages on the photosynthesis of physic nut young plants. Sci Agric 2011;68:62–8.

Silva EN, Vieira SA, Ribeiro RV, Ponte LFA, Ferreira-Silva SL, Silveira JAG. Contrasting physiological responses of *Jatropha curcas* plants to single and combined stresses of salinity and heat. J Plant Growth Regul 2013;32:159–69.

Silveira JAG, Matos JCS, Ceccatto VM, Viégas RA, Oliveira JTA. Nitrate reductase activity, distribution, and response to nitrate in two contrasting *Phaseoulus* species inoculated with *Rhizobium* spp. Environ Exp Bot 2001;46:37–46.

Silveira JAG, Araújo SAM, Lima JPMS, Viégas RA. Roots and leaves display contrasting osmotic adjustment mechanisms in response to NaCl-salinity in *Atriplex nummularia*. Environ Exp Bot 2009;66:1–8.

Sivasankar S, Oaks A. Nitrate assimilation in higher plants: the effect of metabolites and light. Plant Physiol Biochem 1996;34:609–20.

Sun Y, Niu G, Osuna P, Ganjegunte G, Auld D, Zhao L, et al. Seedling emergence, growth, and leaf mineral nutrition of *Ricinus communis* L. cultivars irrigated with saline solution. Ind Crops Prod 2013;49:75–80.

Takahashi S, Murata N. How do environmental stresses accelerate photoinhibition? Trends Plant Sci 2008;13:178–82.

Takahashi S, Badger MR. Photoprotection in plants: a new light on photosystem II damage. Trends Plant Sci 2011;16:53–60.

Weatherburn MW. Phenol-hypochlorite reaction for determination of ammonia. Anal Chem 1967;39:971–4.

Yamori W, Sakata N, Suzuki Y, Shikanai T, Makino A. Cyclic electron flow around photosystem I via chloroplast NAD(P)H dehydrogenase (NDH) complex performs a significant physiological role during photosynthesis and plant growth at low temperature. Plant J 2011;68:966–71.