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Physiological adjustment to salt stress in *R. communis* seedlings is associated with a probable mechanism of osmotic adjustment and a reduction in water lost by transpiration

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ABSTRACT

This study assessed the changes in key physiological processes induced by salinity in *Ricinus communis* seedlings. The experiment was carried out under greenhouse conditions. The results showed that salt stress promoted significant decreases in the dry weight of all the organs studied. Similarly, all the characteristics of leaf gas exchange were significantly reduced with increased NaCl levels. The Na^+ and Cl^- contents were strongly increased in leaves, roots and stems as well as their shoot transport rate, while the concentrations of K^+ and Ca^{2+} in leaves, stems and roots and their transport rates to the shoot were significantly decreased with increasing NaCl levels. In general, our data demonstrate that *R. communis* seedlings present changes in salt stress-induced physiological key responses. These responses are related to stomatal closure and a probable osmotic adjustment mechanism. In this context, the accumulation of Na^+ and Cl^- in leaves and roots played an important role in this osmotic adjustment.

1. Introduction

Salinity is one of the most serious problems that can affect worldwide crop productivity. It can inhibit plant growth through a range of mechanisms, including low external water potential, ion toxicity and interference with the uptake of nutrients, particularly K^+ (Munns and Tester, 2008; Zhang et al., 2010). This problem is most acute in tropical, semi-arid regions, where sodic saline soils are found in large proportions (Ferreira-Silva et al., 2009). In these regions, soil conditions and the climate favor Na^+ accumulation, which causes unfavorable changes in the soil chemical characteristics, such as decreased K^+ availability (Chen et al., 2007; Tammam et al., 2008; Benderradji et al., 2011).

The major saline ions, Na^+ and Cl^-, can affect nutrient uptake through competitive interaction or by affecting membrane selectivity. For example, a high level of Na^+ frequently induces Ca^{2+} and K^+ deficiencies (Tester and Davenport, 2003). High salinity induces both osmotic stress (caused by a high concentration of salt in soil that reduces water uptake by plants) and ionic stress in plant tissues. The latter stress is associated with alterations in the Na^+/K^+ and Na^+/Ca^{2+} ratios due to the accumulation of ions (Na^+ and Cl^-), which can be harmful to plant metabolism (Apse and Blumwald, 2007). In addition, photosynthesis and plant growth are affected by salinity (Munns and Tester, 2008). Salt stress can affect photosynthesis directly or indirectly by decreases in CO_2 availability caused by diffusion limitations (Flexas et al., 2007), alterations in photosynthetic metabolism (Lawlor and Cornic, 2002) or restrictions in the photochemical system apparatus under severe stress conditions (Souza et al., 2004).

Castor (*Ricinus communis* L) is an important oilseed crop because its oil is used to manufacture surfactants, coatings, greases, fungistats, pharmaceuticals, cosmetics and many other products. Castor oil is a promising alternative fuel that can be successfully mixed with petroleum diesel to reduce air pollution (Pinheiro et al., 2008). In addition, this species can be considered a sustainable alternative because it has considerable ability to survive under stressful conditions such as little available nutrition and water (Silva et al., 2008).

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Although some recent reports have demonstrated the effects of salinity on physiological parameters in *R. communis* seedlings (Pinheiro et al., 2008; Li et al., 2010; Janmohammadi et al., 2012; Sun et al., 2013; Lima Neto et al., 2014), an integrative study of growth, leaf gas exchange, transport and selectivity of ions and osmotic adjustment in this species has not been performed. In this context, our aim was evaluate the salt stress-induced changes in key physiological processes in castor seedlings and its capacity to adjust to a saline environment.

2. Materials and methods

2.1. Plant material, growth conditions and harvesting

R. communis seeds of cultivar "Nordestina", kindly provided by the Fitotecnia Department, UFC, Brazil, and previously selected by morphological characteristics such as large size and weight of approximately 0.1 g, were surface sterilized for 1 min with a 5% NaClO solution and placed in plastic trays (30 cm × 40 cm) containing vermiculite to germinate. Twenty days after germination, a group of seedlings was placed into polyethylene trays containing Hoagland and Arnon (1950) nutrient solution (pH maintained at 5.5 using either NaOH or HCl when necessary) at one-fourth strength for 7-days. The aeration system in the nutrient solution was maintained by pumped oxygen.

After 7 days in hydroponic medium, the seedlings were transferred to pots (8 L) and subjected to salt stress for 15 days. The nutrient solution was supplied with 50, 100 or 150 mM NaCl. NaCl was added gradually (25 mmol L^{-1} d^{-1}) into the solution to avoid osmotic shock. The pH was monitored daily. The treatment without NaCl was adopted as a control. The electrical conductivity (EC) of the nutrient solution for the 0, 50, 100 and 150 mM NaCl treatments was 0.5, 5, 10 and 15 dS m^{-1}, respectively. The experiment was carried out in a greenhouse under natural conditions; the mean air temperature varied between 24 °C (minimum) and 36 °C (maximum) with a mean temperature of 29 °C, a mean air relative humidity of 65%, a mean maximum photosynthetic photon flux density (PPFD) of 700 μmol m^{-2} s^{-1} and a photoperiod of approximately 12 h. At the end of the experiment, the seedlings were harvested, and leaves and roots were separated and dried in an oven at 75 °C for 48 h for further chemical analyses.

2.2. Leaf gas exchange

Leaf gas exchange was measured with an infrared gas analyzer-IRGA (LCi, ADC, Hoddesdonm, UK) operating in an open system with an air flow of 200 mL min^{-1}. Measurements of the leaf CO_2 assimilation rate (P_N), transpiration (E), stomatal conductance (g_S) and intercellular CO_2 concentration (C_I) were taken, and the instantaneous carboxylation efficiency (P_N/C_I) was calculated.

2.3. Determinations of ion contents

Oven-dried samples of leaf, root, and stem were soaked in deionized water and placed in a water bath at 100 °C for 1 h. The extracts were then filtered and stored at −20 °C for later analyses. All the tissues were digested before the determinations. The Na^+, K^+ and Ca^{2+} concentrations were determined by an atomic absorption spectrometer (Perkin Elmer Model 5000 USA). The Cl^- concentration was determined through titration with $AgNO_3$ according to the method of Voigt et al. (2009).

2.4. Uptake rates

The transport rates (J_S) of Na^+ (J_{Na}), Cl^- (J_{Cl}), K^+ (J_K) and Ca^{2+} (J_{Ca}) to the shoot were calculated from the changes in the contents of Na^+, Cl^-, K^+, and Ca^{2+} from the onset to the end of the experiment, and they were calculated according to the following equation described by Welbank (1962):

$$J_S = \frac{(M_2 - M_1)}{T_2 - T_1} \times \frac{\ln(W_2 - W_1)}{W_2 - W_1}$$

J_S was calculated after the determination of the ions content in the shoot (mmol kg^{-1} DW) and was expressed on the basis of root dry weight. M_1 and M_2 are the ions content in the shoot (leaf + stem) determined at the onset of the experiment (M_1) and at the end of the experimental period (M_2); $T_2 - T_1$ is the experimental period (15-days); and $W_2 - W_1$ is the difference between the root dry weight at the end (W_2) and at the beginning of the experimental period (W_1). The results are expressed as mmol (kg root DW day)$^{-1}$.

The shoot and root K^+ selectivity in relation to Na^+ ($S_{K,Na}$) were calculated as described by Jeschke and Stelter (1983), as follows:

$$S_{K,Na} = \frac{J_K}{J_{Na}} \times \frac{[Na^+]_{ext}}{[K^+]_{ext}}$$

J_K and J_{Na} represent the K^+ and Na^+ transport rates, respectively, in a specific organ, and $[Na^+]_{ext}$ and $[K^+]_{ext}$ correspond to the Na^+ and K^+ concentrations in the nutrient solution, respectively. The selectivity was expressed in terms of mmol $mmol^{-1}$ (dimensionless).

2.5. Osmolality and relative contribution of each ion to the osmotic adjustment

Small segments from fully expanded leaves and 5 cm-segments of terminal roots were macerated in a mortar. After extract filtration in a miracloth membrane, the sap was centrifuged at 10,000 × g for 10 min at 4 °C. The resultant supernatant was used to determine the osmolality (c) with a vapor pressure osmometer (Vapro 5520, Wescor, USA). The relative contribution (RC) of each ion to the osmotic potential was estimated as the percentage of the osmolality calculated by the following ratio: RC = solute concentration (mmol kg^{-1} water tissue)/osmolality (mmol kg^{-1} solvent) as in Silveira et al. (2009).

2.6. Physiological response analysis (PRA) and statistical analysis

The physiological responses analysis (PRA) to salinity for each trait and all the traits together was calculated by the difference between the minimum and the maximum mean values of the control and stress treatments divided by the maximum mean value as performed in previous studies (Valladares et al., 2000; Balaguer et al., 2001).

The experiment followed a completely randomized design, with four treatments (0, 50, 100 and 150 mM NaCl) and four replications. The experimental unit was one plant in one pot. The data were subjected to analysis of variance, and the means were compared by Tukey's test at the 0.05 confidence level.

3. Results

3.1. Effect of salt stress on growth and leaf gas exchange

Salt stress significantly reduced ($p = 0.05$) the growth of *R. communis* seedlings. The dry weight (DW) of leaves and stems decreased by 35% and 16%, respectively, in plants exposed to mild stress (50 mM NaCl) when compared to the control. However, root dry weight was not affected by this NaCl level (Table 1). Conversely, when the plants were subjected to severe salt stress (100 and 150 mM NaCl), the dry weight was strongly decreased in all the organs studied. The shoot/root ratio was reduced by 21% under mild stress (50 mM NaCl) and was slightly increased in severe stress