

ORGANIC AND INORGANIC SOLUTES AND ITS CONTRIBUTION TO THE OSMOTIC ADJUSTMENT OF WATER STRESSED *Jatropha curcas* PLANTS

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Abstract: The study aimed to evaluate the organic and inorganic solute accumulation and their role about osmotic adjustment (OA) in water stressed *Jatropha curcas* plants. Eight day-old plants had been grown under greenhouse conditions then exposed to water stress treatments, for ten days. The treatments were imposed to well-watered plants and consisted of withholding water supply to get water contents around root zone of 70 (control), 56, 43, 26 and 14% of the substrate holding capacity, meaning 50, 40, 30, 20 and 10 cm³ cm⁻³ substrate water. On treatments, the leaf Ψ_w reduced from -0.55 MPa (control) to -1.05 MPa (10 cm³ cm⁻³ substrate moisture). The K⁺ involvement to the osmotic adjustment was high in both organs as did Na⁺ and Cl⁻. Of the organic solutes studied, TSS has contributed more to the OA in both organs followed by total free amino acids (TFAA) and by glycinebetaine (GB). The data evidence that *J. curcas* plants deal with water stress and mechanisms underlying this may be due good leaf water status and high inorganic and organic solutes concentration in both studied organs.

Key words: water stress, substrate moisture, water status.

Introduction

Drought is the major factor limiting crop productivity world-wide (Chimenti et al., 2006) and crops with increased resistance to this stress appear to be crucial for keeping yield in areas where dry seasons are common. Water stress decreases plant growth and productivity, by slowing the rate of cell division and expansion mainly due to loss of turgor, which results in a decline of the water status components of the plant cells. Relative water content (RWC) as well as water potential (Ψ_w) and its components, turgor potential (Ψ_t) and osmotic potential (Ψ_s), are the parameters most commonly used to assess plant water status (Kiane et al., 2007).

Jatropha curcas is distributed in arid and semi arid areas of South America and all tropical regions. *J. curcas* recently received tremendous attention for its seed oil that can be converted in biodiesel and is considered to be a universally acceptable energy crop (Kumar et al., 2008). It is reported that the species grows in areas with extreme climates and soil conditions that could not be habited by most of the agriculturally important plant species (Francis et al., 2005). However, the physiological mechanisms that control its water stress resistance are scarcely known. Therefore, the aim of present study was to evaluate the organic and inorganic solute accumulation and the relative contribution of them on the osmotic adjustment of *Jatropha curcas* plants submitted to differently substrate moisture levels.

Materials and Methods

Jatropha curcas L. seeds gently provided by the Instituto Fazenda Tamanduá, Brazil, had previously been selected for size and weight, followed by surface sterilization for 1 minute with 5% sodium hypochlorite solution and germinated in sand. Eight days after germination, a homogeneous group of seedlings in height and having the same morphological aspect was transplanted into plastic pots (2 L) filled with vermiculite and remained there for 15 d. One seedling was maintained in each pot. Every two days the pots were watered with one half strength Hoagland and Arnon solution (1950) in quantities enough to bring substrate holding capacity to 70%. The study was carried out in a greenhouse with the following environmental conditions: 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 $\mu\text{mol m}^{-2} \text{s}^{-1}$; and 12 h photoperiod.

In order to have a wide range of water availability to 23-d-old seedlings (\pm eight leaves) a regime from well watered to severe water stress was imposed to them. To do it, the pots were transferred from the greenhouse into a growth chamber. The environmental conditions in the growth chamber were: PPFD of 400

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$\mu\text{mol m}^{-2} \text{s}^{-1}$, air temperature of 27 °C, air humidity of 65% and photoperiod of 12 h. The water stress was progressively induced to the plants by withholding water supply until water content of the substrate reached 70% (control), 56%, 43%, 26% and 14% of its holding capacity, corresponding to approximately 50, 40, 30, 20 and 10 $\text{cm}^3 \text{cm}^{-3}$ of substrate moisture, time at which experiment begun and finished ten days later. During experiment all the pots were daily weighed and corrected for water loss with full strength Hoagland and Arnon (1950) solution, when need. At the end of experiment the plants were harvested, divided into leaves and roots, then frozen and stored at -80 °C for lyophilization and for further chemical and biochemical determinations.

Results and Discussion

Although, increase in concentrations triggered by water deficits have not been commonly found in the tissue of higher plants (Ianucci et al., 2002), our data point out inorganic solutes to be effectively involved in the OA of *Jatropha curcas* plants, especially K^+ , Na^+ and Cl^- , in the leaves and roots (Figure 1). The concentration of K^+ in these two organs was high and its relative contribution to the osmotic potential of *Jatropha curcas* plants under water stress was greater compared with other inorganic ions. K^+ ion is known to be quite soluble and to play a key osmoregulatory role in the guard cells and similarly in turgor maintenance (Taiz and Zeiger, 2006).

Yet, the current results are in line with Patakas et al. (2002) who observed an increase in concentrations of Na^+ and Cl^- in leaves in grapevines under water stress, however Pérez et al. (2009) demonstrated a negligible participation of Na^+ and Cl^- in the osmotic adjustment in lemon plants submitted to drought. An increase in concentration of NO_3^- content (Figure 1) comes probably from inhibition of reductase activity result which has often been observed in many species even under mild stress (Kameli et al., 1995). Although the NO_3^- contribution to the osmotic adjustment have not been as greater as the other organic solutes its relative participation to the osmotic potential can be crucial in both organs in the untreated and treated plants.

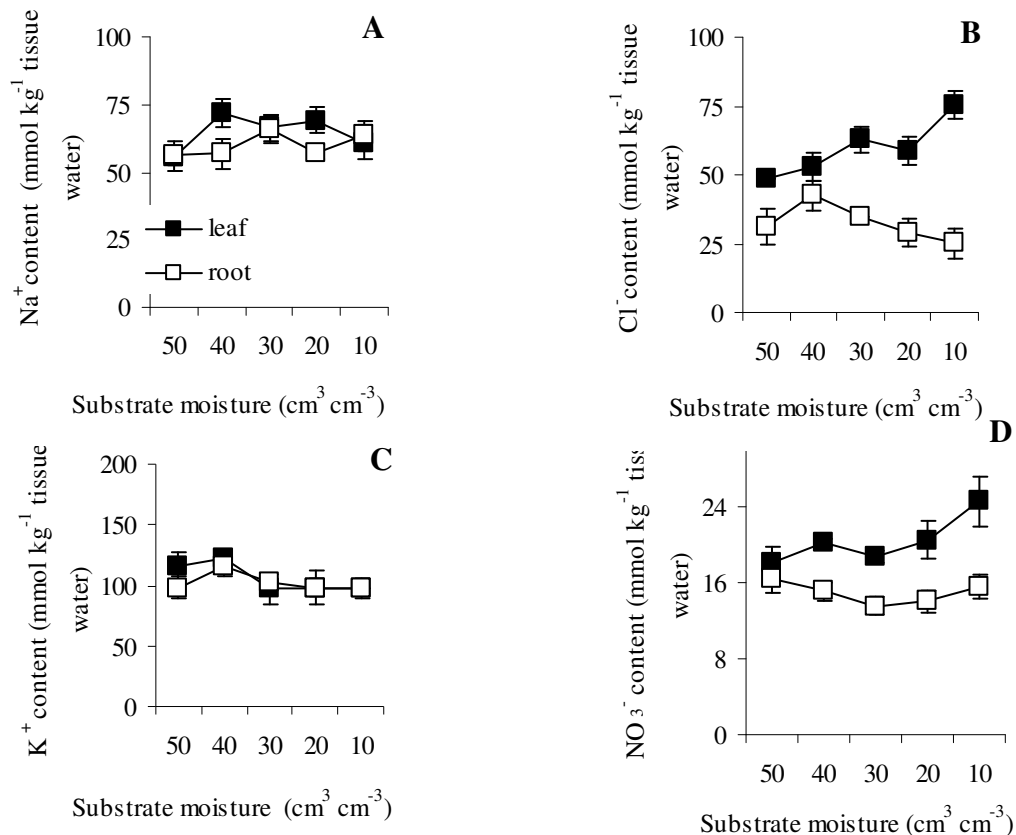


Figure 1. (A) Na^+ content (B) Cl^- content (C) K^+ content and (D) NO_3^- content in the leaves (■—■) and roots (□—□) of *Jatropha curcas* plants exposed to drought different levels. Data are means of four replicates \pm SD.

The organic solutes also had an effective participation to the osmotic adjustment as in the leaves as in roots of *J. curcas* plants, principally TSS, TFAA and glycinebetaine (Figure 2). The increase in the leaf TFAA contents in the more severe drought treatments (20 and 10 cm³ cm⁻³ of substrate moisture) is indicative of a major protein degradation that has been evidenced with a reduction in proteins content (data not shown). On the other hand, the leaf and root TSS contents increasing in all treatments is the result from a decreasing in the utilization of assimilates induced by water stress rather than an inhibitory effect from sucrose synthase or invertase activities (Sturm and Tang, 1999).

Although proline content has not changed in the roots, in the leaves, proline was greater accumulated in the more severe drought treatment. In spite of this, the leaf proline content had not a significant effect in the osmotic potential in *Jatropha curcas* plants. It has been suggested that proline may have different roles in drought mechanism like being involved in the scavenging of free radicals, thereby protecting cellular structures against oxidative damage, and can also serve as a carbon and nitrogen reserve for growth after stress relief (Hare et al., 1998).

In spite of *J. curcas* plants have not undergone changes in the leaf and root GB levels both in control and stressed plants under middle water stress, under the more severe drought treatment the GB content in both organs far increased. There are circumstantial evidences that water stress induced GB synthesis is an adaptive response since it may function as a non-toxic osmolyte or an osmoprotectant that occurs primarily in the cytoplasm (Bajji et al., 2001).

Whether GB accumulation is exclusively confined into cell cytosol that usually makes up 10% of total cell volume, then its contribution in the OA can be 10-fold higher in all studied treatments. In this case, GB would contribute with most OA in the cytoplasm of leaf and root cells. Besides to be considered an efficient osmolyte, GB is thought to improve tolerance to dehydration (Sakamoto and Murata, 2002), to stabilize the protein structure of PSII complex and to prevent damages in the cell membranes of drought stressed plants (Yang et al., 2007). In this context, our data adds weight to the suggestion that GB may have a central role in both cellular protection and cytosol OA in leaf and root cell of *Jatropha curcas* exposed to water stress.

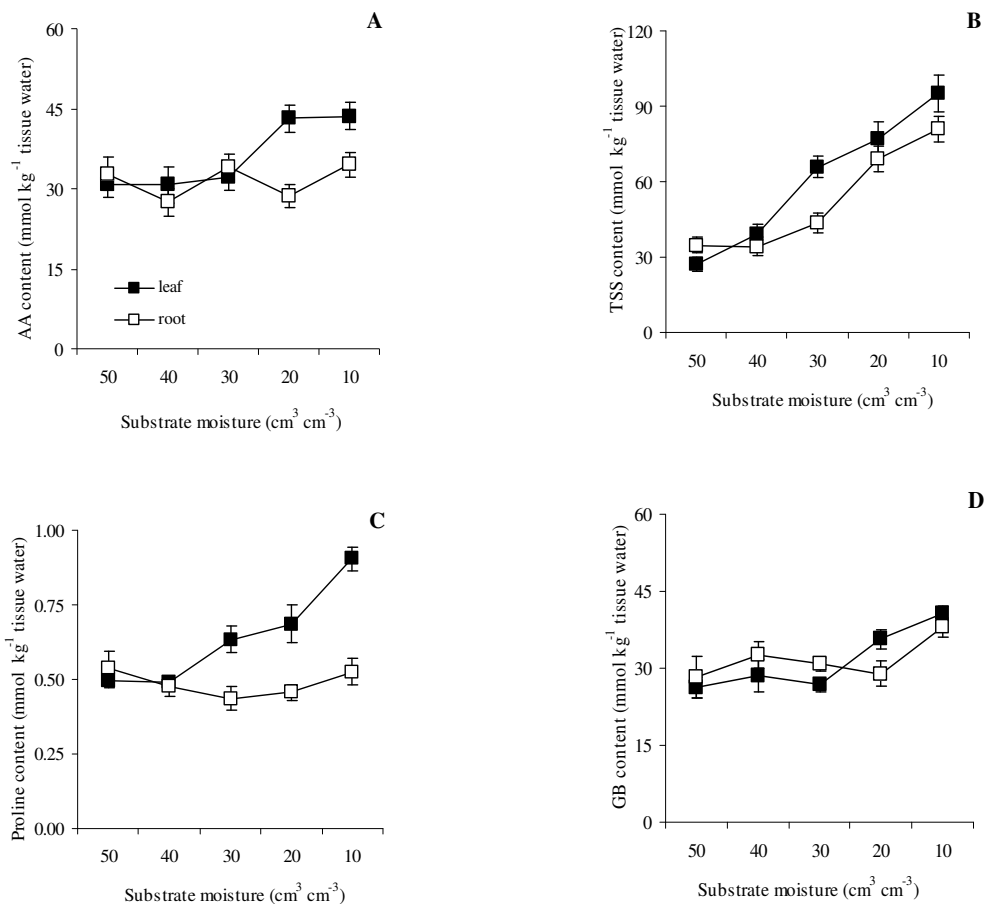


Figure 2. Concentrations of amino acids (A), total soluble sugar (B), proline (C) and glycinebetaine (D) in the leaves (■—■) and roots (□—□) of *Jatropha curcas* plants exposed to drought different levels. Data are means of four replicates \pm SD.

Conclusion

Our findings suggest that *Jatropha curcas* plants osmotic adjustment to drought is mainly carried out by the K^+ and TSS high accumulation in both organs. Together, these compounds make up 40% of the total osmotic adjustment in the leaves and in the roots in all situations tested (control and stress). Others solutes, such as Na^+ , Cl^- , TFAA and GB also are directly involved in drought tolerance in *Jatropha curcas* plants. On the other hand, the leaf and root proline content has not been great enough for contributing with the osmotic adjustment in *J. curcas* plants.

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