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Roots and leaves display contrasting osmotic adjustment mechanisms in response to NaCl-salinity in *Atriplex nummularia*

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ABSTRACT

This study reports contrasting mechanisms between the osmotic adjustment of roots and leaves from a typical halophytic species (*Atriplex nummularia* L.), in response to a large salinity range, resultant from the contribution of inorganic and organic solutes. Plants were grown in a greenhouse and irrigated with nutrient solution containing 0, 75, 150, 300, 450 and 600 mM NaCl during 7 weeks. The maximum leaf and root dry matter accumulation was observed at 300 and 150 mM treatments, respectively. The $Na^+ + Cl^-$ concentrations in leaves were several times higher than in roots (ca. 760 and 90 mM in basis of tissue water, respectively at 300 mM NaCl treatment). Similar tendency was observed in the concentration of the most important organic solute involved with the osmotic adjustment, the glycinebetaine. The other analyzed solutes (K^+, amino acids, soluble sugars and proline) also presented remarkably higher concentrations in leaves compared to roots, in all treatments. As a consequence, the leaf Ψ_s was several times more negative than was in roots. Moreover, the osmotic adjustment of salt-treated plant leaves was approximately 3-fold higher than that found in roots. Surprisingly, under very high levels of external NaCl (450 and 600 mM), the root tissues exhibited Ψ_s values less negative than those found in the external solution. The $Na^+ + Cl^-$ were the major components to the leaves OA followed by K^+ and GB, even in the untreated plants. In contrast to GB, the K^+ participation in both leaves and roots decreased as the NaCl dose increased. In roots, K^+ was the most important solute to OA of salt-untreated plants. Our data evidence that despite the *A. nummularia* leaves displayed an efficient osmotic adjustment, even under very high salinity levels, the same was not observed in its roots, which exhibit high Ψ_s values. This fact probably complicates the root osmotic and water homeostasis in relation to the external medium. We can also conclude that glycinebetaine, followed by soluble sugars, plays a major role in the cytosol osmotic adjustment of both roots and leaves.

1. Introduction

The halophytes are the unique plants able to grow under salt concentrations nearly that of seawater (Winicov and Bastola, 1997; Volkmar et al., 1998). The *Atriplex* genus has several plant species that are capable to complete their life cycles under very harmful environmental conditions such as drought, high temperature and high salinity (Ramos et al., 2004). *Atriplex nummularia* L., commonly named saltbush, is a halophytic C4 species that naturally occurs in the Australian desert regions (Osmond et al., 1980). Halophytic plants are tolerant to salinity in part because they are able to uptake water by maintaining a high osmotic potential through the accumulation of inorganic and organic solutes (Bradley and Morris, 1991; Winicov and Bastola, 1997). Several species belonging to the genus *Atriplex* are adapted to harsh environmental conditions and therefore constitute a useful material for the identification of physiological mechanisms and genes involved in abiotic stress resistance (Cabello-Hurtado and Ramos, 2004; Wang and Showalter, 2004; Hassine et al., 2008).

Although the halophytes plants display marked differences in their salt tolerance degree (Glenn et al., 1999), they share a similar salt inclusion strategy to deal with excessive salinity (Flowers et al., 1977). In this process, the leaf tissues are adapted to accumulate large amounts of saline ions, sometimes greater than those surrounding roots. Such adaptive mechanism is crucial to generate a water potential gradient along root–shoot in order to maintain

Abbreviations: GB, glycinebetaine; OA, osmotic adjustment; RWC, relative water content; TSS, total soluble sugars; TFAA, total free amino acids; DM, dry matter.

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water flux throughout plant. Moreover, the protection of cytosolic structures and biomolecules in some halophytic plants is made by sequestering saline ions into vacuoles of specialized cells, termed trichomes and vesicles, which are located in the leaf epidermis (Glenn et al., 1999). These specialized cells are believed to be very efficient to exclude toxic ions from cytosol as well as contribute to the overall osmotic adjustment of leaf cells.

The OA is an adaptive mechanism that might contribute to drought tolerance mechanisms in plants and it results from net accumulation of solutes in cells in response to decrease in the water potential or excess salt in the environment (Chimenti et al., 2002; Wang et al., 2003). Most studies involving the Na^+ ion accumulation in *Atriplex* species focalize on the saline conditions and there is a general acceptance that this ion is very important for total OA, especially when it is available in high amounts in the external medium (Storey and Wyn Jones, 1978; Osmond et al., 1980). Na^+ contribution to OA is not always direct but may also occur indirectly. Indeed, Subbarao et al. (2001) demonstrated that Na^+ may trigger GB synthesis independent on osmotic stress. According to Glenn and Brown (1998), tolerance to water and salt stresses in *A. canescens* are linked through a common mechanism of Na^+ uptake which is directly used for OA. In *A. halimus*, the real contribution of Na^+ to OA is difficult to quantify precisely at the whole plant level, since a considerable part of Na^+ accumulates in trichomes covering the leaf surface (Mozafar and Goodin, 1970).

Ionic homoeostasis and osmotic adjustment (OA) of cytosol appears to be mediated by increased synthesis of organic protectants, especially proline (Pro) and glycinebetaine (GB), that are preferentially located in the cytosol (Moghaieb et al., 2004). Glycinebetaine is virtually absent in some important crop species such as rice and tomato, but it accumulates in high amounts under salt or water stress conditions in plants belonging to the family of Chenopodiaceae (Wyn Jones and Storey, 1981; McCue and Hanson, 1990). This is especially the case of the genus *Atriplex*; for example, *A. hortensis* has successfully been used as a source of the coding gene for betaine aldehyde dehydrogenase that converts betaine aldehyde into glycinebetaine for transgenic approaches in rice (Guo et al., 1997), tobacco (Shen et al., 2002), or tomato (Jia et al., 2002). In Chenopodiaceae species, such as those belonging to the genus *Atriplex*, the cytosol OA is thought to be mainly due to great accumulation of GB, which may also assume positive functions in relation to the maintenance of membrane integrity and stability of other cellular structures under water-stress conditions (Shen et al., 2002; Wang and Showalter, 2004). Recent experimental evidence has demonstrated that plant species able to synthesize GB may also accumulate other organic compatible solutes, such as Pro, and that the kinetics of accumulation of these compounds depends on the stress intensity and the duration of stress exposure (Di Martino et al., 2003).

The K^+ ion plays a central role in OA, turgor maintenance, and in the stomata opening control of plants under physiological or stress conditions (Maathuis and Amtmann, 1999). The mechanisms by which K^+ acts in the OA and in the ionic homeostasis of cytosol, in both halophytes and glycophytes are not adequately elucidated yet, as well as the processes involved with the interaction of Na^+ with K^+, particularly in relation to membrane selectivity (El-Haddad and O'Leary, 1994). Furthermore, K^+ is very important to the cytosol ionic homeostasis maintenance in Na^+-stressed plants (Zhu, 2003). The salt stress often causes reduction in the plant tissues K^+ content, especially in roots, through mechanisms not completely understood yet (Viégas et al., 2001). The specialization of some *Atriplex* species for saline habitats could involve peculiar abilities to accumulate both K^+ and Na^+ ions (Ramos et al., 2004). The general model proposed to ionic and osmotic cellular homeostasis in leaf cells of halophytes is presented through a balance of inorganic and organic solutes compartmentalized in cytosol and vacuoles. The saline ions (Na^+, Cl^-) and a minor fraction of K^+ are rather sequestrated into vacuoles whereas most K^+ and virtually all the organic osmo-solutes are confined in the cytosol (Flowers et al., 1977).

Most studies on salinity tolerance in halophytes consider OA resulting from either inorganic solutes (Naidoo and von Willert, 1995; Ramos et al., 2004) or organic solutes (Poljakoff-Mayber et al., 1987), but they rarely comprise both. Besides, the majority of the published works on osmotic adjustment of halophytes are carried out with leaves and scarcely with roots. Thus, comprehensive studies which have involved the simultaneous participation of both inorganic and organic solutes in the osmotic adjustment of roots and leaves of typical halophytes species are scarce. Some works have pointed out that in some halophytes under high salinity the leaf Ψ_s is several times more negative than in roots (Bajji et al., 1998; Ramos et al., 2004). However, these works did not propose any explanation on the mechanisms underlying those responses. Thus, despite the immense amount of studies involving halophyte species there is clearly a gap concerning to the mechanisms involved on the osmotic adjustment of roots as well as on the involvement of the organic and inorganic solutes, especially under long-term exposure to a large range of salinity.

In the present work, the hypothesis that leaves and roots of *A. nummularia* display distinct mechanisms to undergo osmotic adjustment under a vast range of NaCl-salinity was tested. The relative contribution of the major inorganic (Na^+, Cl^- and K^+) and organic (glycine betaine, proline, amino acids and sugars) solutes in both organs was compared. The contribution of these solutes in the osmotic homeostasis of root and leaf cells is discussed.

2. Material and methods

2.1. Plant material and growth conditions

A. nummularia L. (saltbush) was propagated through terminal herbaceous stem segments (15 cm long), excised from plants cultivated under field conditions of a semi-arid region of Brazil. The rooting was carried out in 2 l polyethylene bags, containing sand as a substrate. After 60 days, the plants were transplanted to 5 l plastic pots containing the same propagation media, where they remained for further 60 days. In both cases, the plants were daily watered with 120 ml of one-half strength Hoagland and Arnon's solution (1950), and were grown in a greenhouse under natural conditions with 28/24 °C day/night average temperatures, 55/85% air relative humidity, average maximum photosynthetically active radiation density at plant canopy of approximately 700 μmol m^{-2} s^{-1} and a 12 h photoperiod.

2.2. Treatments and plant harvest

A homogeneous group of 120-day-old plants was selected for experiment. The plants were irrigated by one step with one-half strength Hoagland and Arnon's solution supplemented with 0; 75; 150; 300; 450 and 600 mM NaCl. The plants irrigated with nutrient solution with absence of NaCl were taken as control. All the pots were irrigated every 2 days with a volume of 250 ml per pot (approximately 70% of soil holding capacity). Once at each week, the pots were saturated and the percolated solution collected for electrical conductivity and osmotic potential determinations. This latter was performed in a vapor pressure osmometer (Vapro 5520, Wescor, USA) and the average values were: −0.12; −0.52; −0.83; −1.45; −2.31; and −2.93 MPa, corresponding to 0; 75; 150; 300; 450 and 600 mM NaCl treatments, respectively. Every week, the pots received a sufficient volume of distilled water to leach the root media and to avoid salt accumulation. The plants were harvested after 7 weeks of treatments. Before the plant harvest, intact leaves (thirty per plant) from the shoot middle part (main stems, with