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Effects of short- and long-term salinity on leaf water relations, gas exchange, and growth in *Ipomoea pes-caprae*

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ABSTRACT

Ipomoea pes-caprae is widespread in pantropical coastal areas along the beach. The aim of this study was to investigate the salinity tolerance level and physiological mechanisms that allow *I. pes-caprae* to endure abrupt increases in salinity under brief or prolonged exposure to salinity variations. Xylem sap osmolality (X_{osm}), leaf water relations, gas exchange, and number of produced and dead leaves were measured at short- (1–7 d) and long- (22–46 d) term after a sudden increase in soil salinity of 0, 85, 170, and 255 mM NaCl. In the short-term, X_{osm} was not affected by salinity, but in the long-term there was a significant increase in plants grown in presence of salt compared with control plants. After salt addition, the plants showed osmotic stress with temporal cell turgor loss. However, the water potential gradient for water uptake was re-established at 4, 7 and 22 d after salt addition, at 85, 170 and 255 mM NaCl, respectively. In the short-term *I. pes-caprae* was able to tolerate salinities of up to 255 mM NaCl without significant reduction in carbon assimilation or growth. With the duration of stress, leaf ion concentration continued to increase and reached toxic levels at high salinity with a progressive decrease in photosynthetic rate, reduced leaf formation and accelerated senescence. Then, if high levels of soil salts from tidal inundation occur for short periods, the survival of *I. pes-caprae* is possible, but prolonged exposure to salinity may induce metabolic damage and reduce drastically the plant growth.

Introduction

In coastal sandy habitats, the proximity to the coast and the seasonal distribution of rainfall result in pronounced variation in soil salinity and fresh water availability. Substrates continuously receive high levels of salt from aerosol spray and oceanic waters, and typically experience salinities oscillating from fresh to seawater (Bowman and Strain, 1988; Howard and Mendelssohn, 1999; Jefferies et al., 1979; Omer and Barclay, 2002). In this environment, a range of higher plants with contrasting ecophysiological properties co-exist and are often classified as halophytes or non-halophytes with different levels of salt tolerance. In the former, growth is stimulated by intermediate salt levels and they are found exclusively in saline soils, whereas in the latter, low shoot ion accumulation generally inhibits plant growth and they are typically more widespread in distribution (Ahmed and Khan, 2010; Flowers et al., 1986; Greenway and Munns, 1980; Gul et al., 2001; Jennings, 1976; Naidoo and Rughunanan, 1990). Some non-halophyte species are able to occupy saline coastal habitats due to the fact that they are deep rooted and able to utilize fresh groundwater (Meloni et al., 2008; Ripley and Pammenter, 2004). Nevertheless, during initial development phases, when roots are superficial, all species occupying coastal sandy environments are exposed to sudden increases and fluctuations in soil salinity (Meloni et al., 2008).

In species that experience abrupt increases in soil salinity, selection probably has acted upon physiological traits that favor tolerance to rapidly imposed salinity. These probably include rapid osmotic adjustment and recovery of photosynthetic capacity following tidal inundation (Akhiyarova et al., 2005; Bowman and Strain, 1988; Flowers et al., 1986). In some salt marsh and crop species, this adjustment to seawater salinity can be achieved in 24–48 h (Akhiyarova et al., 2005; Bowman and Strain, 1988; Flowers et al., 1986; McNulty, 1985; Murphy et al., 2003). Fast osmotic adjustments require ion accumulation up to a concentration equal to or greater than that of the surrounding root solution in order to achieve an osmotic gradient for the uptake of soil water (Flowers et al., 1986; Greenway and Munns, 1980; Naidoo and Rughunanan, 1990). However, at long-term and high salinity, salt uptake by roots must be reduced in order to prevent toxic accumulation of ions (Flowers et al., 1986; Greenway and Munns, 1980; Rozema and Van Diggelen, 1991). Controlled inorganic ion uptake must be accompanied by ion compartmentation in the vacuoles and by the synthesis and accumulation of compatible solutes (Flowers et al., 1986; Greenway and Munns, 1980; Munns and

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Tester, 2008). Additionally, a decrease in stomatal conductance and transpiration rate is likely to be the first plant defence against increases in soil salinity (Gale and Poljakoff-Mayber, 1970; Gul et al., 2001; Redondo-Gómez et al., 2007; Robinson et al., 1997). Leaf gas exchange is generally reduced when soil salinity increases rapidly as a consequence of a reduction in stomatal conductance, but in the long-term some metabolic determinants of photosynthesis may also be inhibited (Akhiyarova et al., 2005; Flowers et al., 1986; Redondo-Gómez et al., 2007; Seemann and Critchley, 1985). Salinity also may affect plant growth. At short-term, growth inhibition can be explained by reduced leaf elongation through decrease in turgor of cell expanding tissues, while at long-term salinity affects many different aspects of growth, including those associated with water stress and those specific to NaCl (Akhiyarova et al., 2005; Greenway and Munns, 1980; Munns and Termaat, 1986; Ramani et al., 2006; Thiel et al., 1988). Thus, when salinity tolerance and physiological responses are evaluated in species that have evolved under naturally occurring salinity stress, both the intensity and the time course over which the stress is imposed should be considered (Bowman and Strain, 1988; Flowers et al., 1986; Howard and Mendelssohn, 1999).

This study was undertaken to investigate the salt tolerance in *Ipomoea pes-caprae* (L.) R. Br. (Convolvulaceae) under conditions in which salinity is rapidly increased and maintained for relatively short or long periods under controlled conditions. *I. pes-caprae* is a perennial beach plant that forms large patches just above the high tide line on coastal beaches and dunes, where it is exposed to salt-spray and inundation by either salt or fresh water (Devall, 1992; Ripley and Pammenter, 2004). In typical habitats these inundations can occur frequently and it can be relatively permanent or temporal, depending on the variability of the local precipitation and the tidal regime (Martínez et al., 2002). *I. pes-caprae* has been considered a halophyte plant (Omer and Barclay, 2002; Venkatesan and Chellappan, 1998), a facultative halophyte (Devall, 1992; Medina et al., 2008) and also a non-halophyte (Ripley and Pammenter, 2004). Thus, short- and long-term salinity tolerance and the physiological mechanisms that allow *I. pes-caprae* to endure rapid increases in soil salinity were evaluated. Additionally, with this information it is possible to answer whether *I. pes-caprae* is actually a halophytic species and its degree of tolerance. Xylem sap osmolality, water relation parameters and leaf gas exchange were measured at short-term, the first seven days, and at long-term at 22 and 46 d after salt addition at four levels of moderate salinity in the nutrient solution.

Material and methods

Plant material and cultivation

Ipomoea pes-caprae (L.) R. Br. (Convolvulaceae) is a clonal, perennial herbaceous species with a pantropical distribution widespread in coastal areas, and one of the earliest species to colonise newly deposited dunes (Devall, 1992; Ripley and Pammenter, 2004). Plants, growing in sand along the beach and above the high tide line, were collected in Tucacas, Estado Falcón, Venezuela (10°48′N, 68°19′W) and transported to a glasshouse facility at Universidad Simón Bolívar, Caracas, Venezuela (10°25′N, 66°50′W). Twenty-eight apical vegetative segments of uniform size, with four to seven leaves, were transplanted into 5-L pots filled with sand and watered as needed with *Peat-Lite Special* Peters® Water Soluble Fertilizers (Scotts Company Marysville, OH) plus an additional supply of $Ca(NO_3)_2$. Plants were kept in the glasshouse under natural sunlight, with a 12 h photoperiod. The maximum photosynthetic photon flux density was $1550 \pm 370\ \mu mol\ m^{-2}\ s^{-1}$; air temperature was 25–35 °C during the day and 15–20 °C at night, and the relative air humidity ranged between 49 and 97%. Before beginning the experiment, apical segments were grown for ten months to allow recovery from transplantation and to allow root formation. All plants survived. After this period, the plants were irrigated with nutrient solution containing 0, 85, 170, and 255 mM NaCl until the free substrate drainage reached these concentrations, to obtain four salinity treatments for eight plants each. Marine salt (98% NaCl) was preferred to pure NaCl as the source of salinity as it simulates the effect of salinity under natural conditions. The salinity of the nutrient solutions was measured with a refractometer, and water solution osmolality (W_{osm}) was measured using a Wescor 5500 vapor pressure osmometer (Wescor Inc., Logan, UT, USA). Based on these data, it was adjusted every day during the first five days of treatments and every three days thereafter.

Salt was added at night (7:00 p.m.) the previous day to the beginning of measurements, and salinity levels were maintained during the experimental period. Measurements were carried out thereafter during the following seven days and at 22 and 46 d after salt addition. During the days 1–7 of salt addition (short-term) it is assumed that the plants are in a phase of adjustment to salinity in the nutrient solution, while at 22 and 46 d after salt addition (long-term) the plants are in a steady state, as indicated by Flowers et al. (1986) and Munns and Tester (2008). It is important to note that the length and intensity of salt exposure used in this study could have been beyond the tolerance limits of the species, and that under natural conditions the intensity and duration of salt exposure depends markedly of the local characteristics.

Measurements

Xylem sap samples were collected in four branches from different plants per treatment, at predawn, to ensure that the xylem sap was near equilibrium with soil water. The leaf petiole was placed in a pressure chamber and pressure was increased until water flowed from the cut end. The initial exudate was eliminated because of mixing with the latex characteristic of *I. pes-caprae*. The xylem exudate was collected on filter paper and the osmolality (X_{osm}) measured using a vapor pressure osmometer. The xylem sap osmotic potential was calculated using the Van't Hoff equation: $\Psi_\pi = -cRT\rho$, where, R is the gas constant, T the Kelvin temperature, c the osmolality of the xylem sap, and ρ the density of water.

Leaf water potential (Ψ_w) was determined at predawn, in four plants per treatment, with a pressure chamber (Model 1400, PMS Instruments Co., Corvallis, OR, USA). After determination of Ψ_w, leaves were rinsed with distilled water and dried with tissue paper. One-half of the leaf blades, excluding the middle veins, were placed in plastic syringes, immediately frozen in liquid nitrogen, and stored until leaf sap osmotic potential (Ψ_π) was measured. The frozen samples were thawed for 1/2 h at room temperature before leaf sap osmolality determination with a vapor pressure osmometer. Thereafter, Ψ_π was calculated using the Van't Hoff equation. Predawn turgor potential (Ψ_t) was calculated by subtracting Ψ_π from Ψ_w. The other leaf blade halves were used to determine the relative water content (RWC), sampling 10 disks of 7 mm diameter. The fresh mass (M_f) of the disk was measured immediately, and then re-hydrated to full turgidity for 4 h under normal room light and temperature by floating in distilled water in a covered Petri dish. After that, samples were taken out of the water and dried quickly and lightly of any surface moisture with filter paper, and immediately weighed to obtain their fully turgid mass (M_t). The samples were then oven dried for 48 h at 70 °C to determine dry mass (M_d). RWC was calculated as: $RWC = (M_f - M_d)/(M_t - M_d) \times 100$. Dry mass per leaf area (M_d/A) was calculated and leaf succulence was estimated as water content ($W_c = M_f - M_d$) expressed on a leaf area basis.