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Sesuvium portulacastrum maintains adequate gas exchange, pigment composition, and thylakoid proteins under moderate and high salinity

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ABSTRACT

Cuttings of *Sesuvium portulacastrum* L. (Aizoaceae) were taken from plants cultivated under severe saline conditions. The obtained seedlings were grown on sand and irrigated with nutrient solution over 5 weeks under no (0 mM NaCl), moderate (200 mM NaCl), or high (400 mM NaCl) salinity conditions. A follow-up of gas exchange was performed weekly and pigment levels and patterns of fully expanded leaves were determined after 3 and 5 weeks of treatment. At the end of the 5-week period, immunoblot analysis of the main polypeptides of photosystem I and II was performed with the aim to investigate salt-induced variations in photosystem composition.

Net CO_2 assimilation rate (*Pn*) increased under salinity up to 3 weeks of treatment then decreased to reach the value of 0 mM-treated plants at the end of the experiment. For stomatal conductance (*gs*) and intercellular CO_2 concentration (*Ci*), the opposite occurred. These results were concomitant with an increase in practically all pigment levels, mainly under high salinity, with the exception of zeaxanthin. The de-epoxidation index (DEPS index) was much lower under saline than non-saline conditions in the 3rd week, indicating light stress in 0 mM-treated plants. At the end of the experiment, this index showed much lower values with no significant differences between treatments, which coincided with no significant differences in gas exchange as well. Protein amounts of D1, CP47, and CP43 did not show noticeable variations with salt treatment, whereas LHCII underwent a slight but significant decrease (−15%) at the highest NaCl concentration. LHCI polypeptides were unaffected by the salt treatments, where conversely, the highest concentration induced a significant decrease in PsaA/B amount (−18%).

Introduction

Salinity is a major environmental factor that affects plant growth and crop yields (Munns, 2002). This is due to a low osmotic potential of soil solution (osmotic stress), specific ion effects (salt stress), nutritional imbalances, or a combination of these factors (Ashraf, 1994; Zhu, 2002). All of these factors induce detrimental effects on plant physiology and biochemistry (Munns, 2002). Nevertheless, based on their capacity or lack of capacity to grow in highly saline environments, plants are traditionally classified as halophytes and glycophytes, respectively (Türkan and Demiral, 2009). Halophytes are plants able to achieve their life cycle in environments where salt concentration is around 200 mM NaCl or more. They represent 1% of the world flora, with the remaining 99% represented by glycophytes (Flowers and Colmer, 2008). Some halophytes show optimal growth under saline conditions, whereas others grow optimally under non-saline conditions (Flowers and Colmer, 2008).

Salt stress is known to reduce net CO_2 assimilation rate (*Pn*), transpiration rate (*E*), and stomatal conductance (*gs*) in glycophytes (Ouerghi et al., 2000; Gibberd et al., 2002; Burman et al., 2003; López-Climent et al., 2008) and some halophytes (Nieva et al., 1999; Liao and Guizhu, 2007). Salt-induced effects on photosynthesis can be attributed to a limitation of stomatal conductance (Brugnoli and Björkman, 1992; Goldstein et al., 1996), non-stomatal limitations (Drew et al., 1990; Belkhodja et al., 1999), or both, depending on tissue salt concentration, with stomatal closure occurring at low salt concentration followed by a reduction of photosynthesis activity when salt concentration surpasses a certain threshold (Downton et al., 1990). Photosynthesis limitation can be also attributed to inhibiting feedback exerted by high sugar concentrations in mesophyll cells often observed in leaves of salt-treated plants. This excessive sugar accumulation is due to disequilibrium in their use

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within developing tissues (Munns et al., 1982). Salt tolerance in plants is therefore related to their aptitude to maintain adequate *Pn* and *gs* (Lakshmi et al., 1996) and a high chlorophyll level (Krishna Raj et al., 1993) under saline conditions. The chlorophyll response to salinity seems to depend on stress severity. Low salinities generally lead to an increase in chlorophyll levels (Locy et al., 1996), whereas severe salinities often reduce them (Malibari et al., 1993). In some other halophytes, photosynthesis has been shown to be unaffected by salinity, or even stimulated at low salt concentrations (Rajesh et al., 1998; Kurban et al., 1999; Parida et al., 2004). Venkatesalu and Chellappan (1993) studied photosynthesis in the C_3 halophyte *Sesuvium portulacastrum* L., at different salinity levels (from 0 to 900 mM NaCl) over 6 months of treatment, aiming to investigate whether this species could experience a shift in its photosynthesis pathway. They noted an improvement in *Pn* and *gs*, with a maximum at 600 mM NaCl, but no shift from C_3 to C_4 or CAM metabolism.

S. portulacastrum was described by Lonard and Judd (1997) as an important pioneer species on sandy beaches in the tropics and subtropics. They attributed its success as a colonizing species, in large part, to its ability to be propagated by salt-tolerant vegetative fragments as well as to its tolerance of salt spray, sand scouring and burial, high substrate temperatures, and low soil nutrient concentrations. *S. portulacastrum* is used as food, fodder (Ramani et al., 2006; Lokhande et al., 2009), and as an essential oil source (Magwa et al., 2006). It is also used in ornamentation, landscaping, desert greening, and sand dune fixation (Messedi et al., 2001; Lonard and Judd, 1997). Ghnaya et al. (2005) also showed its capacity as a hyperaccumulator of heavy metals. In addition, it was found to be efficient in bioreclamation of salt-affected soils as a result of its aptitude to produce high biomass and to accumulate enormous sodium quantities within its shoots (Ravindran et al., 2007; Rabhi et al., 2009). Lokhande et al. (2009) reported its use as an alternative culture to problematic soils. Hence, *S. portulacastrum* could be a good candidate for the recently established approach suggesting the use of selected domesticated halophytes to overcome salinity problem (Lieth et al., 1999). Although many studies of the photosynthetic responses of this species to salinity have been performed, some features still require elucidation. The aim of this investigation was to examine whether the maintenance (or even the enhancement) of photosynthetic activity under salinity is due to the maintenance of chloroplast integrity. Pigment levels and patterns and photosystem protein composition of fully expanded leaves were investigated at different salinity levels.

Materials and methods

Plant material and treatments

Cuttings of *Sesuvium portulacastrum* were taken from mother plants grown under greenhouse conditions at 400 mM NaCl. During a 3-week period of rooting, the cuttings were irrigated with non-saline tap water. Thereafter, seedlings of about 5 cm height were transferred onto sand and irrigated with Hewitt's (1966) nutrient solution added (200 or 400 mM) or not (0 mM) with NaCl. The plant culture was carried out under greenhouse conditions and lasted 5 weeks.

Gas exchange analysis

Photosynthetic performance in fully expanded leaves was measured weekly. Gas exchange measurements were performed for net CO_2 assimilation rate (*Pn*), stomatal conductance (*gs*), and intercellular CO_2 concentration (*Ci*) at 10.00–12.00 am. All measurements were carried out in the greenhouse at saturating light ($800\ \mu mol\ m^{-2}\ s^{-1}$ over the PAR waveband) using a portable infrared gas analyzer Li-Cor 6400 (Li-Cor Inc., Lincoln, NE, USA) operating at 35 ± 0.5 Pa ambient CO_2.

Leaf pigment analysis

At the end of the 3rd and 5th weeks of treatment, the same leaves used for gas exchange measurements were harvested, immediately frozen in liquid nitrogen, and stored at −80 °C for pigment analyses. Pigment level and pattern were determined according to the method reported by Castagna et al. (2001). Frozen samples were homogenized in the dark in 100% HPLC-grade acetone with 1 mM sodium ascorbate then filtered through 0.2 μm filters. The analysis was performed by HPLC using a Zorbax ODS column (Chrompack SA, 5-m particle size, Ø250 mm × 4.6 mm) for pigment separation. Two solvents were used: A (acetonitrile/methanol, 75/25, v/v) and B (methanol/ethylacetate, 68/32, v/v).

The separation cycle was 32 min with a flow rate of $1\ mL\ min^{-1}$. Pigments were eluted using 100% A for the first 15 min, followed by a 2.5-min linear gradient to 100% B, which continued isocratically until the end of the cycle. The column was allowed to re-equilibrate in 100% solvent A for 10 min before the next injection. Pigments were detected by their absorbance at 445 nm, and their quantification was performed by the injection of known amounts of pure standard into the HPLC system and the formulation of an equation correlating peak area to pigment level. The latter was expressed as $nmol\ g^{-1}$ FW.

Thylakoid isolation and immunoelectrophoretic analysis of their proteins

After 5 weeks of treatment, fully expanded leaves were harvested and kept in the dark at 4 °C for 30 min. Then, they were homogenized in a grinder using a grinding buffer (1/10, w/v) containing 330 mM sorbitol, 15 mM NaCl, 5 mM $MgCl_2$, 20 mM Na-ascorbate, 10 mM $NaHCO_3$, 10 mM Na_2-EDTA, 0.1% (w/v) bovine serum albumine, and 50 mM Tricine-NaOH (pH 7.8). The homogenization was performed through three pulses (3 s per pulse) to avoid temperature increase. The obtained homogenate was filtered through 16 layers of sterile gauze and centrifuged at 7000 × *g* for 30 s. The resulting pellet was resuspended in shocking buffer (15 mM NaCl, 5 mM $MgCl_2$, 50 mM HEPES-NaOH, pH 7.2), kept on ice in the dark for 10 min and then centrifuged at 5000 × *g* for 10 min. The pellet was finally resuspended with the shocking buffer containing 100 mM sorbitol and 20% sucrose. After that, it was frozen in liquid nitrogen and stored at −80 °C until immunoelectrophoretic analysis (modified from Ranieri et al., 1997). Chlorophyll level was calculated according to Lichtenthaler (1987).

For immunoblotting analysis, different amounts of thylakoid membranes from each treatment, corresponding to 0.25, 0.50, and 1.00 μg total chlorophyll, were dissolved in 2% SDS, 4 M urea, 611 mM β-mercaptoethanol, and 2.75 mM Tris–HCl, pH 6.8, kept at 60 °C for 30 min then loaded on adjacent lanes of 15% SDS-urea polyacrylamide gels according to the method of Ranieri et al. (2000). After electrophoretic separation by a Bio-Rad Mini-Protean II® device, proteins were transferred from gels to nitrocellulose membranes and then tested by antisera polyclonal antibodies against spinach D1, CP47, CP43, LHCII and PsaA/PsaB, and against maize LHCI polypeptides. Antigen–antibody interactions were revealed using goat antirabbit IgG biotin conjugate and avidine-alkaline phosphatase conjugate (SIGMA, St. Louis, MO, USA), in the presence of the chromogenic substrates nitro blue tetrazolium (NBT) and 5-bromo,4-chloro,3-indolyl phosphate (BCIP) following the method of Ranieri et al. (1997). Quantification of band intensity was performed using Kodak DIGITAL SCIENCE 1D software.