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South African Journal of Botany 79 (2012) 39–47

SOUTH AFRICAN JOURNAL OF BOTANY

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Photosynthetic responses to salinity in two obligate halophytes: *Sesuvium portulacastrum* and *Tecticornia indica*

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Received 27 May 2011; received in revised form 23 September 2011; accepted 15 November 2011

Abstract

Seedlings of the obligate halophytes *Sesuvium portulacastrum* L. and *Tecticornia indica* (Willd.) subsp. *indica* were grown with 0, 200, or 400 mM NaCl for 13 weeks to investigate whether salt tolerance was related to maintenance of adequate photosynthetic activity and pigment equipment. Both species showed growth optimum at 200 mM NaCl and better tissue hydration under salinity but different photosynthetic response to salinity. CO_2 assimilation rate and stomatal conductance of *S. portulacastrum* were highest at 200 mM NaCl, while in *T. indica* they decreased with salinity. Pigment content increased under salinity in both species. The de-epoxidation state in *S. portulacastrum* suggests the need for energy dissipation at 400 mM NaCl, while its salt-induced decline in *T. indica*, despite the reduced photochemistry, suggests the involvement of adaptive mechanisms other than the xanthophyll cycle.

Keywords: Carotenoids; Chlorophyll; Photosynthesis; Salt stress

1. Introduction

Salinity is one of the major environmental constraints which affects plant productivity and crop yields worldwide (Munns, 2002). Such an effect is often associated to salt-induced reduction in photosynthetic capacity, as salt stress impairs net CO_2 assimilation rate (P_N), transpiration rate (E), and stomatal conductance (g_s) (Chaves et al., 2009; Gibberd et al., 2002; Koyro, 2006; Lakshmi et al., 1996; Sudhir and Murthy, 2004). Chlorophyll response to the stress depends on its severity; low salt concentrations generally increase its content (Locy et al., 1996), whereas severe salinity often reduces it (Malibari et al., 1993). Salt-induced photosynthesis reduction can be attributed to a limitation in stomatal conductance (Brugnoli and Björkman, 1992; Goldstein et al., 1996), a non-stomatal limitation (Drew et al., 1990), or both, with a diminution of stomatal conductance at low salt contents in tissues then a reduction in photosynthesis activity when their salt content reaches a certain level (Downton et al., 1990). Some studies showed a negative correlation between gas exchange and leaf Na^+ and Cl^- concentrations (Gibberd et al., 2002). A disturbance in ion acquisition can also lead to a decline in K^+ content in chloroplasts and disintegration of photosystem II (PSII) (Solomon et al., 1994). Photosynthesis limitation can be also attributed to an inhibiting «feedback» exerted by high sugar content in mesophyll cells

Abbreviations: A, antheraxanthin; C_i, intercellular CO_2 concentration; Chl, chlorophyll; DEPS index, De-epoxidation index; DM, dry mass; E, transpiration rate; g_s, stomatal conductance; P_N, net CO_2 assimilation rate; PSII, photosystem II; RGR, relative growth rate; SI, salt sensitivity index; V, violaxanthin; VAZ pigments, sum of violaxanthin, antheraxanthin, and zeaxanthin; *WUE*, water use efficiency; Z, zeaxanthin; ΔDM, dry mass difference between the beginning and the end of the experiment.

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doi:10.1016/j.sajb.2011.11.007

often observed in leaves of salt-treated plants. This excessive sugar accumulation is due to disequilibrium in its use within developing tissues (Munns et al., 1982). Salt tolerance in plants is therefore related to a plant's aptitude to maintain adequate P_N and g_s (Lakshmi et al., 1996) and high chlorophyll content (Krishna Raj et al., 1993) under saline conditions.

In some other studies, photosynthesis was shown not to be affected by salinity and even stimulated at low salt concentrations (Kurban et al., 1999; Parida et al., 2004; Rajesh et al., 1998). Generally, such photosynthetic adaptation to salinity is observed in halophytes, which are plants able to survive and to reproduce in environments where the salt concentration is around 200 mM NaCl or more (Flowers and Colmer, 2008). Nevertheless, differences in photosynthetic responses to salinity exist even between obligate halophytes that are species requiring salt for an optimal biomass production. For instance, the obligate halophyte *Sesuvium portulacastrum* (Aizoaceae) displayed an improvement in photosynthesis rate and stomatal conductance in response to salinity (from 0 to 900 mM NaCl), with a maximum at 600 mM NaCl (Venkatesalu and Chellappan, 1993). In the same species, we observed time-dependent fluctuations in net CO_2 assimilation rate under salinity, with an increase up to 3 weeks of treatment followed by a decrease to the value of untreated plants after 5 weeks (Rabhi et al., 2010). Conversely, Nieva et al. (1999) found that salinity negatively affected photosynthesis in the two obligate halophytes *Arthrocnemum perenne* and *A. fruticosum* (both belonging to the family of Chenopodiaceae).

Akhani et al. (1997) reported that the chenopod *Tecticornia indica* (Willd.) subsp. *indica* belongs to the C_4 group (this species is also called *Artrocnemum indicum* (Willd.) Moq., *Salicornia indica* Willd., and *Halosarcia indica* (Willd.) Paul G. Wilson). It was shown to be a NAD-malic enzyme-type C_4 species, with mesophyll chloroplasts having reduced grana, characteristic of this subtype (Voznesenskaya et al., 2008). This species displayed Kranz anatomy with reduced leaves and fleshy stem cortex. Kranz anatomy produces spatial separation of atmospheric CO_2 fixation in the mesophyll cells from the fixation of the CO_2 released from C_4 acids in the bundle sheath cells. This avoids CO_2 leakage from the mesophyll to the atmosphere and maintains an adequate CO_2/O_2 ratio at the Rubisco site in the bundle sheath (Murchie et al., 2009).

S. portulacastrum and *T. indica* were shown to exhibit a variety of interests. *S. portulacastrum* is used as food, fodder (Lokhande et al., 2009: Ramani et al., 2006), and essential oil source (Magwa et al., 2006). It is also used in ornamentation, landscaping, desert greening, and sand dune fixation (Lonard and Judd, 1997). Ghnaya et al. (2005) proved also its capacity as a hyperaccumulator of heavy metals. Lokhande et al. (2009) reported also its use as an alternative culture to problematic soils. *T. indica* subsp. *indica* is considered as a potential oilseed plant (Weber et al., 2007) and as useful fodder appreciated by dromedary, livestock, and occasionally cattle (Le Houérou and Ionesco, 1973). In saline ecosystems, the tufts of this perennial species offer to several annual glycophytes a favorable microhabitat and considerably contribute to the annual productivity (Abdelly et al., 1995). Actually, *T. indica* as well as *S. portulacastrum* are efficient plants in phytodesalination of salt-affected soils thanks to their high aptitude to produce biomass and to accumulate enormous quantities of sodium within their shoots (Rabhi et al., 2009; Ravindran et al., 2007). Estimations of Rabhi et al. (2009) showed that these two succulent halophytes accumulated respectively 2507 and 711 kg sodium per hectare in 170 days when grown on a saline soil (ECe = 19 dS m^{-1}) taken from borders of a salt marsh. Hence, these two species can be good candidates for the recently-established approach suggesting the use of selected domesticated halophytes to overcome the problem of salinity (Lieth et al., 1999).

Differences in the ability to adapt to a changing environment have been reported between C_3 and C_4 plants, which account for their competitive colonization of different habitats. Plants with C_4 photosynthesis have advantages in extreme growth conditions such as high temperature, low water availability, high irradiation or saline soils (Sage, 2004, and references within). High light favors C_4 plants because of reduced bundle sheath leakage of CO_2 under high irradiance (Henderson et al., 1992; Kubásek et al., 2007; Tazoe et al., 2008). Due to the temperature-dependence of photorespiration, the quantum yield of C_3 plants declines as temperature increases, so that C_4 metabolism takes a competitive advantage at warm temperature (Ehleringer et al., 1997; Osborne et al., 2008). The predicted increase in ambient CO_2 concentration is expected to favor C_3 photosynthesis (Ehleringer et al., 1997), although a recent paper reports that combined CO_2 enrichment and global warming led to increased above-ground growth of C_4 grasses in semi-arid grasslands (Morgan et al., 2011).

In the present research, we investigated whether the ability of the halophyte species *T. indica* and *S. portulacastrum* to tolerate high salt concentrations within their shoots was related to the maintenance of adequate photosynthetic activity and leaf pigment contents and patterns. To this aim, plants were grown for 13 weeks in order to move from the study of plant responses to acute stress conditions towards a more realistic scenario, with plants allowed to adapt to the different salt regimens.

2. Materials and methods

2.1. Plant culture and determination of growth and photosynthesis parameters

After rooting of *Sesuvium portulacastrum* and *Tecticornia indica* cuttings, seedlings of about 50 mm height (and 2 leaves in the case of *S. portulacastrum*) were transferred onto sand and irrigated with Hewitt's (1966) nutrient solution added (200 or 400 mM) or not (0 mM) with NaCl. Plants were grown for 13 weeks from October 2007 to January 2008 under greenhouse conditions at a day/night temperature of 22/10 °C, an average daily relative humidity of 70–90%, a light intensity of 180–200 μmol photons m^{-2} s^{-1} PAR provided by fluorescent L58W/77 lamps (Osram, München, Germany), and a photoperiod of 10–12 h. Just before harvest, photosynthetic performance was measured. The same leaves (*S. portulacastrum*) and internodes (*T. indica*) on which photosynthetic parameters were determined were harvested then immediately frozen in liquid nitrogen

and stored at −80 °C for pigment analyses. The remaining intact plants were harvested, cut into shoots and roots, weighed, and oven-dried for dry mass and sodium content determination.

2.2. Gas exchange

Leaf gas exchange measurements were conducted for net CO_2 assimilation rate (P_N), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i). All measurements were carried out in the greenhouse at 800 μmol m^{-2} s^{-1} PAR waveband using a CO_2/H_2O-porometer (Li-Cor 6400, Li-Cor Inc., Lincoln, USA) operating at 35±0.5 Pa ambient CO_2. Leaves (*S. portulacastrum*) and internodes (*T. indica*) were numbered starting from the plant apex and measurements were performed for fully expanded middle-aged leaves or internodes at 10.00–12.00 h at a measuring chamber temperature of 35 °C.

2.3. Leaf pigment analysis

Pigment concentrations and pattern of *S. portulacastrum* leaves and *T. indica* internodes were determined according to the method reported by Castagna et al. (2001). Frozen samples were homogenized in dark in 100% HPLC-grade acetone with 1 mM sodium ascorbate then filtered through 0.2-μm filters. The analysis was performed by HPLC (HPLC P200, Thermo Fisher Scientific, Waltham, MA, USA) using a non-endcapped column (Zorbax ODS column, Chrompack, Raritan, NJ, USA) 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 1920s with a flow rate of 16.67 mm^3 s^{-1}. Pigments were eluted using 100% A for the first 900s, followed by a 150-s 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 600 s before the next injection.

Pigments were detected by their absorbance at 445 nm, and their quantification was realized by the injection of known amounts of pure standard into the HPLC system and the formulation of an equation correlating peak area to pigment concentration. The latter was expressed as nmol g^{-1} DM.

2.4. Sodium contents

After oven-drying, root and shoot samples were ground then digested with a HNO_3 (0.5%) solution for ion extraction. Their sodium contents were determined by a flame photometer (Corning 480, Corning Medical and Scientific. Ltd., Halstead, England).

2.5. Calculated parameters

ΔDM Biomass production during the treatment period is the quantity of dry matter produced between the beginning and the end of the treatment period. It was determined according to the following equation: $\Delta DM = DM_2 - DM_1$.

RGR Relative growth rate measures how much biomass is producing each gram of existing biomass at an instant t. It was calculated according to Hunt's (1990) equation:

$$RGR = [\ln DM_2 - \ln DM_1]/[t_2 - t_1].$$

SI Salt sensitivity index measures variations in dry mass between salt-treated (200 or 400 mM NaCl) and non-treated (0 mM NaCl) plants. It was determined as follows:

$$SI = 100*[\Delta DM_{\text{salt treatment}} - \Delta DM_{0\text{ mM NaCl}}]/\Delta DM_{0\text{ mM NaCl}}$$

In these three equations, DM — the total plant dry mass (mg); t — time (d); the subscripts 1 and 2 — the initial and final harvests.

WUE Water use efficiency is defined as the net carbon uptake per amount of water lost from a transpiring leaf area. It was calculated as the ratio of net photosynthesis rate (P_N) and transpiration rate (E): $WUE = P_N/E$ (Koyro, 2006).

DEPS index De-epoxidation index calculation was based on the contents of antheraxanthin (A), zeaxanthin (Z), and violaxanthin (V) according to the following equation:

$$\text{DEPS index} = [(A/2) + Z]/[V + A + Z].$$

2.6. Statistical analysis

Data were subjected to a one-way ANOVA test using SPSS software (SPSS 11.0 for Windows, IBM company, Inc., Chicago, USA) and means were compared according to *Duncan*'s test at $P = 0.05$.

3. Results

3.1. Biomass production and tissue hydration

Plants of *T. indica* and *S. portulacastrum* showed an increase in biomass production when grown under saline conditions (Table 1). Of the three salt concentrations used in this experiment, 200 mM seems to be the optimum for their shoot biomass (Table 1). At this salt concentration, shoot dry mass reached 154 and 132%, respectively in *T. indica* and *S. portulacastrum*. A thirteen-week cultivation at 400 mM NaCl resulted in a significant decrease in shoot biomass production as compared to the 0 mM NaCl treatment in *S. portulacastrum*, whereas in *T. indica*, no significant difference was observed between 0 and 400 mM NaCl. However, when compared to the optimal salt concentration (200 mM NaCl), shoot biomass underwent a similar

Table 1
Shoot and root dry mass (DM) and water content, relative growth rate (RGR), dry mass difference between the beginning and the end of the experiment (ΔDM), and sensitivity index (SI) calculated on the basis of whole plant DM, in *Sesuvium portulacastrum* and *Tecticornia indica* plants grown under greenhouse conditions at 0, 200, and 400 mM NaCl for 13 weeks. Values represent the mean of 12 determinations. For each species, values of the same line followed by different letters are significantly different according to *Duncan*'s test ($P=0.05$).

NaCl (mM)	*Sesuvium portulacastrum*			*Tecticornia indica*		
	0	200	400	0	200	400
Shoot DM (g plant^{-1})	4.67^{b}	6.18^{c}	3.24^{a}	1.21^{a}	2.01^{b}	1.21^{a}
Root DM (g plant^{-1})	0.59^{b}	0.84^{c}	0.28^{a}	0.35^{b}	0.38^{b}	0.21^{a}
Root/shoot ratio	0.13^{b}	0.14^{c}	0.09^{a}	0.31^{b}	0.19^{a}	0.19^{a}
Shoot H_2O (mm^3 mg^{-1} DM)	5.88^{a}	11.28^{b}	11.80^{b}	4.92^{a}	7.98^{b}	7.83^{b}
Root H_2O (mm^3 mg^{-1} DM)	4.26^{a}	7.63^{b}	5.55^{a}	3.38^{a}	6.70^{b}	7.76^{b}
ΔDM (mg lant^{-1})	5.01^{b}	5.90^{b}	3.40^{a}	1.46^{a}	2.29^{b}	1.33^{a}
RGR (d^{-1})	0.028^{a}	0.036^{c}	0.031^{b}	0.025^{a}	0.029^{b}	0.024^{a}
SI (%)	-	32.3	−30.6	-	66.1	0.0

decrease in both halophytes (−48% and −40% in *S. portulacastrum* and *T. indica*, respectively).

Plant cultivation with 200 mM NaCl stimulated root growth in *S. portulacastrum*, as indicated by the 42% increase in root biomass, but not in *T. indica*, while the high salinity level (400 mM NaCl) drastically affected root growth in both species (Table 1). Such a negative effect was higher in *S. portulacastrum* than in *T. indica*, when calculated in respect to both 0 and 200 mM salt concentrations. In this latter case salt stress induced a −67% and −45% decrease in *S. portulacastrum* and *T. indica*, respectively, in comparison to optimal salt conditions (200 mM NaCl).

Despite the diminution of biomass production under high salinity, the two halophytes exhibited better tissue hydration under saline than non-saline conditions in both shoots and roots. No significant differences were observed between the two salt concentrations, the only exception being roots of *S. portulacastrum*, which, instead, showed a lower water content at 400 mM than at 200 mM NaCl (Table 1). As far as shoots are concerned, at 400 mM water content reached 160% of the 0 mM NaCl treatment in *T. indica* and 201% in *S. portulacastrum*.

3.2. Sodium accumulation

As grown under saline conditions (200 and 400 mM NaCl), the two halophytes showed much higher sodium contents in shoots than in roots (Fig. 1). Roots exhibited a continuous increase with increasing salinity, although differences between 200 and 400 mM NaCl treatments were not statistically significant in *T. indica* (Fig. 1C, D). In shoots however values were similar for the two salt treatments, indicating a threshold of sodium accumulation within their tissues (7.9 and 9.2 mmol $Na^+ g^{-1}$ DM, respectively in *T. indica* and *S. portulacastrum*; Fig. 1A, B).

3.3. Gas exchange and stomata responses

In the 0 mM NaCl treatment, P_N of *S. portulacastrum* plants (9.3 μmol CO_2 m^{-2} s^{-1}) was two-fold that of *T. indica* (4.7 μmol CO_2 m^{-2} s^{-1}; Fig. 2A, B). Under saline conditions, the two halophytes showed different photosynthetic responses. In *S. portulacastrum*, P_N was enhanced at 200 mM NaCl and reached 148% of the 0 mM NaCl treatment, while at 400 mM NaCl it diminished back to the values recorded at 0 mM NaCl. In *T. indica*, this parameter did not show significant differences between 0 mM and 200 mM NaCl, but it underwent a −70% reduction at 400 mM NaCl. Similarly to P_N, g_s was higher in *S. portulacastrum* than in *T. indica* under non-saline conditions (Fig. 2C, D). In addition, g_s followed the same tendency as P_N in *S. portulacastrum*, with an optimum at 200 mM NaCl, while it showed a continuous decrease with salinity in *T. indica*. C_i did not noticeably vary with salt concentration in *S. portulacastrum* and the highest values were observed at 200 mM NaCl (Fig. 2E). In *T. indica*, however, C_i was reduced under salinity although differences were not statistically significant (Fig. 2F). The overall tendency of *WUE* under salinity showed a decrease in *S. portulacastrum* and an increase in *T. indica* although differences between treatments were not statistically significant in some cases (Fig. 2G, H).

3.4. Leaf pigment content and pattern

Under non-saline conditions, *S. portulacastrum* and *T. indica* showed different total chlorophyll contents, the registered values being 1782 and 2183 nmol g^{-1} DM, respectively (Table 2). Differences in chlorophyll *a*/*b* ratio (Chl *a*/*b* ratio) between the two halophyte species were also found, *T. indica* showing the highest values regardless of the treatment. Under saline conditions, total chlorophyll content was significantly enhanced in both species. However, while in *S. portulacastrum* this stimulation was found to be similar at 200 and 400 mM NaCl (+278% and +243%, respectively), in *T. indica*, chlorophyll content underwent a gradual salt-dependent increase (+61% and +103% at 200 and 400 mM NaCl, respectively; Table 2). Chl *a*/*b* ratio was slightly modified by salinity and, in both species, it underwent significant changes only at 400 mM NaCl, when it was found to increase in *S. portulacastrum* and to decrease in *T. indica*.

Similarly to chlorophyll, carotenoids also showed higher values in *T. indica* than in *S. portulacastrum*. In detail, Table 2 shows that in *T. indica* 0 mM NaCl-treated plants, total carotenoid and xanthophylls were about 1.5-fold those in *S. portulacastrum*. In salt-grown plants, the content of these pigments considerably increased in both species but while in *S. portulacastrum* they reached similar values at 200 and 400 mM NaCl (about +270% and +260% for total carotenoid and xanthophylls, respectively), in *T. indica*, a progressive enhancement of their content was observed (+59% and +57% at 200 mM NaCl and +89% and +85% at 400 mM NaCl, for total carotenoids and total xanthophylls, respectively). The same trend was also followed by β-carotene, lutein, and neoxanthin, while the sum of the three xanthophylls involved in the

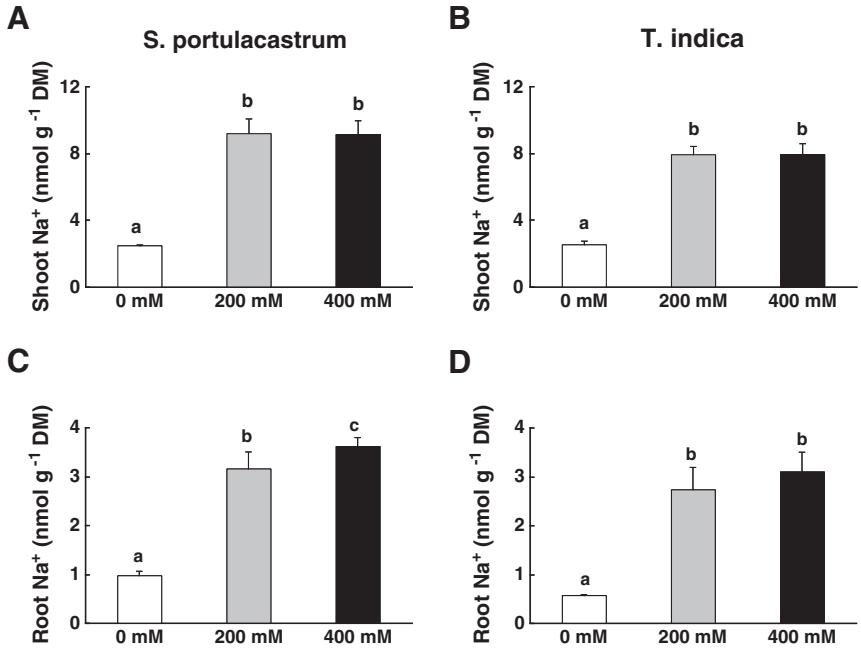

Fig. 1. Shoot (A, B) and root (C, D) sodium contents of *Sesuvium portulacastrum* and *Tecticornia indica* plants grown under greenhouse conditions at 0, 200, and 400 mM NaCl for 13 weeks (mean±SE, n=6). Bars with different letters are significantly different according to *Duncan*'s test at $P=0.05$.

zeaxanthin cycle (VAZ pigments) behaved differently. Indeed, VAZ pigments underwent a gradual noticeable accumulation in *S. portulacastrum* (+182% and +304% at 200 and 400 mM NaCl, respectively) while they increased much less and to a constant value in *T. indica* (+43% and +50% at 200 and 400 mM NaCl, respectively; Table 2). Salt-induced variations in the content of the three single xanthophylls participating in the zeaxanthin cycle led to complex variations in the de-epoxidation (DEPS) index. Actually, in *S. portulacastrum* DEPS index significantly increased over the 0 mM NaCl treatment at 400 mM NaCl (+53%) while it showed the lowest value at 200 mM NaCl. Conversely, in *T. indica*, growth under saline conditions induced a similar, significant decrease in DEPS index at both salt concentrations (−77% and −85% at 200 and 400 mM NaCl, respectively; Table 2).

4. Discussion

According to Yeo and Flowers (1980), the fact that dry mass of dicotyledonous halophytes increases at moderate salinity makes the use of the term "control" for plants grown under non-saline conditions not appropriate since suboptimal salt solutions might formally be described as deficient. For this reason, the 0 mM NaCl treatment was not called "control" in our study.

The C_4 halophyte *T. indica* produced less biomass than *S. portulacastrum* but it allocated more carbon to the roots, as indicated by the higher root/shoot ratio exhibited by *T. indica* at any salt concentration (Table 1). Accordingly, *T. indica* could have a competitive advantage in soil exploiting as compared to *S. portulacastrum*.

Both species exhibited the typical halophyte behavior, as indicated by the increase in biomass accumulation and the higher RGR under moderate salt concentration (200 mM NaCl) as compared to 0 mM NaCl (Table 1). However, in *T. indica* only shoot growth was stimulated by salt addition. At a higher concentration (400 mM NaCl), growth decreased in both species in comparison to the optimal growth conditions (200 mM NaCl), but differences in root and shoot salt sensitivity occurred between the two halophytes. The root/shoot ratio was indeed reduced by high salt concentration in *S. portulacastrum* in comparison to 200 mM NaCl, because of the higher reduction in root than shoot dry mass, but not in *T. indica*, which experienced similar decrease in above- and below-ground biomass (Table 1).

For *S. portulacastrum*, our results are in agreement with those of Messedi et al. (2003) who worked on seedlings taken from the same mother-plants but grown under more controlled conditions. These authors mentioned that *S. portulacastrum* is an obligate halophyte and its growth is stimulated by salt concentrations less than 400 mM NaCl, the limit of its halophytic behavior. As far as *T. indica* is concerned, Nagarajan et al. (2008) found that its growth increased up to 300 mM NaCl and noticeably decreased over this concentration.

In order to better compare the effect of salinity on each halophyte, we calculated their relative growth rates (RGR), biomass production over the 13 weeks of treatment (ΔDM), and their sensitivity index to salinity (SI) (Table 1). RGR variations revealed that under saline as well as non-saline conditions, *S. portulacastrum* showed higher growth rate than *T. indica*. Therefore, taking into account the long period of treatment (13 weeks), the former showed much higher ΔDM values than the latter. But with regard to the 0 mM NaCl treatment of each species, the highest salt concentration (400 mM NaCl) affected *S. portulacastrum* more than *T. indica*. Indeed, the sensitivity indexes to the stress were −30.6% and 0%, respectively

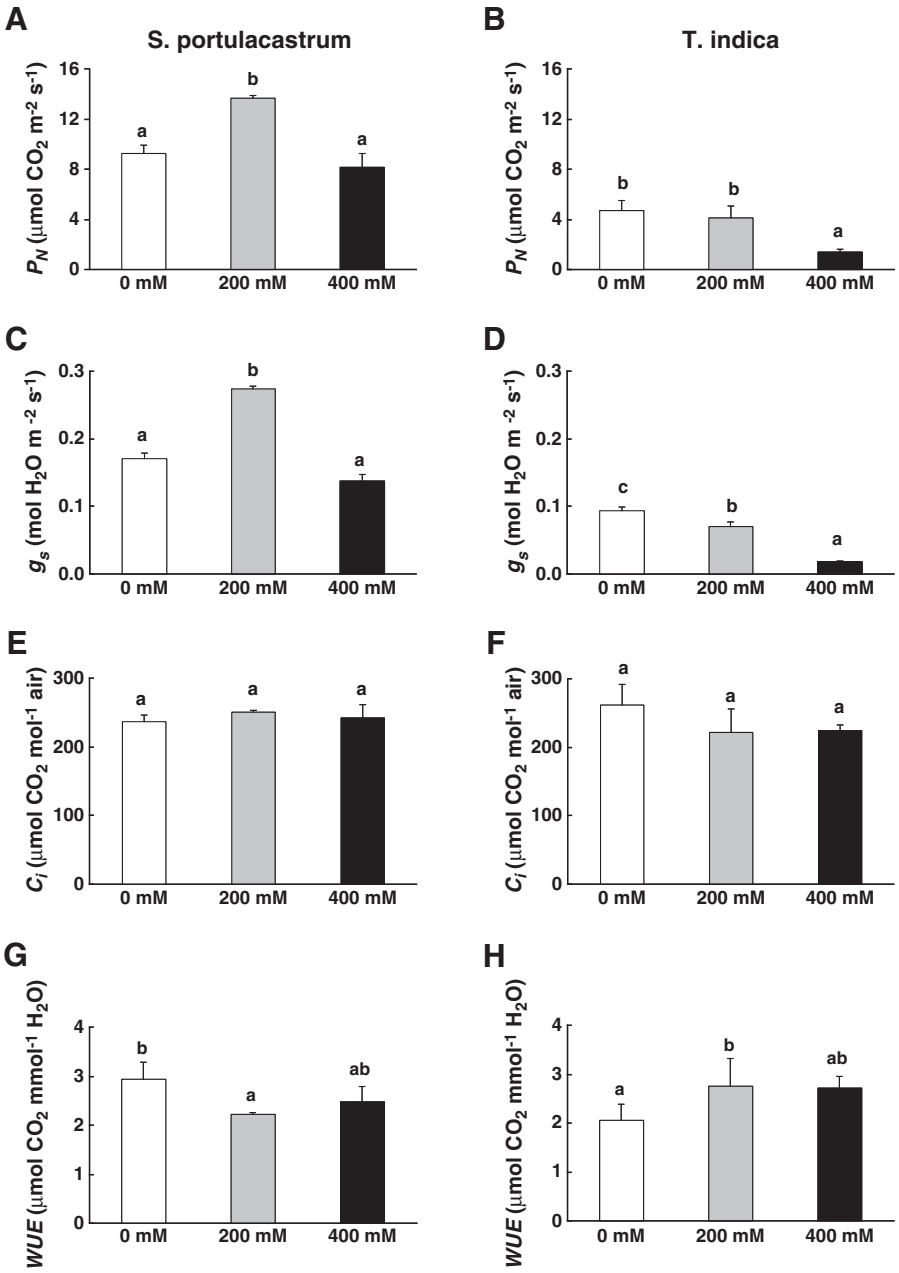

Fig. 2. Net CO_2 assimilation rate (P_N; A, B), stomatal conductance (g_s; C, D), intercellular CO_2 concentration (C_i; E, F) and water use efficiency (*WUE*; G, H) in *S. portulacastrum* and *T. indica* plants grown under greenhouse conditions at 0, 200, and 400 mM NaCl for 13 weeks (mean ± SE, n = 12). Bars with different letters are significantly different according to *Duncan*'s test at $P = 0.05$.

in the two halophytes. The higher sensitivity of *S. portulacastrum* to salt stress in respect to *T. indica* was also confirmed by the SI values calculated in respect to the optimal growth conditions (200 mM NaCl), which were −42.4% and −8.9%, respectively. Conversely, *T. indica* was found to be more sensitive to salt deprivation, as indicated by the SI value more than twice higher (−36.2%) than in *S. portulacastrum* (−15.1%).

P_N was much higher in *S. portulacastrum* than in *T. indica* regardless of the treatment (Fig. 2). This could explain why over the same period, the former produced more biomass than the latter. In *S. portulacastrum*, photosynthetic activity and stomatal conductance followed the same variations as growth, whereas in *T. indica*, the high growth stimulation noticed at 200 mM NaCl coincided with a trend to decrease in P_N and a reduction in g_s. It should be however remembered that photosynthetic performance is highly dependent on light environment. In C_4 plants (as *T. indica*), bundle sheath leakage of CO_2 previously fixed by PEP carboxylase, which is believed to be the main source of variation in photosynthetic efficiency, is greater in low-light than in high-light conditions (Henderson et al., 1992; Kubásek et al., 2007; Tazoe et al., 2008). Moreover, as far as light intensity increases, C_3 carbon fixation

Table 2
Leaf pigment content (nmol g^{-1} DM) and pattern in *Sesuvium portulacastrum* and *Tecticornia indica* plants grown under greenhouse conditions at 0, 200, and 400 mM NaCl for 13 weeks. Values represent the mean of 3 determinations. For each species, values of the same line followed by different letters are significantly different according to *Duncan*'s test ($P=0.05$). Chl — chlorophyll; DEPS index — de-epoxidation index; VAZ pigments — sum of violaxanthin, antheraxanthin and zeaxanthin.

NaCl (mM)	*Sesuvium portulacastrum*			*Tecticornia indica*		
	0	200	400	0	200	400
Chlorophyll *a*	1043^{a}	4001^{b}	3716^{b}	1670^{a}	2677^{b}	3375^{c}
Chlorophyll *b*	739^{a}	2740^{b}	2394^{b}	513^{a}	835^{b}	1060^{c}
Total Chlorophyll	1782^{a}	6741^{b}	6109^{b}	2183^{a}	3511^{b}	4435^{c}
Chl *a*/*b*	1.41^{a}	1.45^{ab}	1.55^{b}	3.26^{b}	3.21^{ab}	3.18^{a}
Lutein	165.9^{a}	644.8^{b}	582.5^{b}	220.3^{a}	365.3^{b}	445.2^{c}
Neoxanthin	32.3^{a}	121.0^{b}	108.9^{b}	50.2^{a}	77.7^{b}	95.4^{c}
Violaxanthin	40.7^{a}	136.7^{b}	123.3^{b}	54.8^{a}	132.8^{b}	149.2^{b}
Anteraxanthin	4.8^{a}	15.0^{a}	33.9^{b}	23.0^{b}	15.9^{ab}	7.6^{a}
Zeaxanthin	11.2^{a}	9.2^{a}	72.9^{b}	31.3^{a}	6.8^{b}	6.1^{b}
VAZ	56.7^{a}	160.9^{b}	230^{c}	109.2^{a}	155.5^{b}	162.9^{b}
Xanthophylls	254.8^{a}	926.6^{b}	921.4^{b}	379.7^{a}	598.5^{b}	703.5^{c}
DEPS index	24.7^{ab}	10.2^{a}	37.8^{b}	39.4^{b}	9.2^{a}	6.0^{a}
β-carotene	84.0^{a}	344.9^{b}	341.7^{b}	163.1^{a}	263.0^{b}	319.4^{c}
Total carotenoids	339^{a}	1272^{b}	1263^{b}	543^{a}	861^{b}	1023^{c}

undergoes light saturation and an increasing proportion of light energy is dissipated as heat. Therefore, in the natural environment where light is higher than that used in the present experiment, a C_4 plant should better perform than a C_3 one.

Actually, the reduction in photosynthetic activity of succulent C_3 plants (such as *S. portulacastrum*) under salinity is usually lower than that of C_4 plants (like *T. indica*) (Drake, 1989). Both species exhibited no variations in C_i when grown under saline conditions. In *S. portulacastrum*, the parallel trend of P_N and g_s, together with unchanged C_i value, clearly indicates that stomatal response was the main determinant for salt-dependent changes in CO_2 assimilation. *S. portulacastrum* has been previously found to underwent fluctuation in net CO_2 assimilation rate under salinity (mainly at 400 mM NaCl) over a 5 week period, with an increase up to 3 weeks of treatment followed by a decrease to the value of untreated plants after 5 weeks. These changes were accompanied by a generally reduced g_s (Rabhi et al., 2010) and suggest the attempt of the photosynthetic apparatus to adapt to the growth environment.

S. portulacastrum and *T. indica* adopted two different growth-photosynthesis relationships. The former exhibited the same behavior towards increasing salinity as the halophyte *Cakile maritima* on the basis of RGR and P_N. The two parameters showed an increase at 100 mM NaCl and a decrease at higher salinity levels, but RGR was less affected by the stress than P_N (Debez et al., 2006). In fact, several controversies characterize this relationship in the literature. The results of *WUE* cannot explain the ability of *T. indica* to maintain a biomass production in salt-treated plants similar to that of the 0 mM NaCl treatment (Table 1) despite the decrease in P_N and g_s under salinity (Fig. 2). *T. indica* must have evolved a specific mechanism that still needs to be elucidated. In its natural biotope, *T. indica* is a well-adapted plant to the severe conditions of a sabkha (more or less flat, shallow depression subjected to periodic inundation and evaporite deposition) during the driest period of the year in which plants have to cope with at least the interactive effects of salinity and drought stresses (Rabhi et al., 2009). In general, Chenopodiaceae are well-adapted to aridity and among 305 studied species, 205 adopt C_4 photosynthetic pathway (Akhani et al., 1997). Shoot sodium content was not responsible for the differences in growth and photosynthesis between *T. indica* and *S. portulacastrum*. Indeed in the two halophytes, at 200 as well as at 400 mM NaCl, it was maintained at a high and constant value (Fig. 1), although growth and photosynthesis responses were completely different. Nevertheless, it seems that by reducing their stomatal conductance at severe salinity, they tend to reduce sodium loading into their shoots and thus help increase their longevity by maintaining Na^+ at subtoxic levels longer than it would occur if g_s was not diminished (Everard et al., 1994). This was especially the case for the C_4 species *T. indica*.

Leaf pigment content was higher in salt-treated plants of both halophytes, but particularly in *S. portulacastrum* (Table 2). Such a generalized higher pigment concentration in both species could suggest an increased number of photosystems induced by growth in a saline environment. Our results agree with those of Venkatesalu and Chellappan (1993) who found that total chlorophyll content was significantly higher in plants grown under saline conditions over 90 as well as 180 days in comparison with the 0 mM NaCl treatment. *S. portulacastrum* leaves exhibited an extremely low Chl *a*/*b* ratio, differently from what recorded by Ramani et al. (2006), who measured higher values of Chl *a*/*b* ratio accompanied by an increase in such a ratio after a short-term salt treatment. Since low light conditions are known to decrease the ratio between the two chlorophylls, the differences observed between Ramani's report and our data may be attributed to the different light environment. The present experiment was indeed carried out at a lower light intensity than that used by Ramani and co-workers. A low Chl *a*/*b* ratio could be related to an increase of antenna size with respect to core complexes (CCs), chlorophyll *b* being not associated with CC polypeptides, to maximize light harvesting.

The increase in Chl *a*/*b* ratio under salinity, observed in *S. portulacastrum* grown at 400 mM NaCl (Table 2), is in agreement with the finding of Ramani et al. (2006) and suggests a rearrangement of the photosystem composition in order to avoid the risks of photoinhibition. *S. portulacastrum* plants grown for 5 weeks at 400 mM NaCl were indeed found to display a decreased amount of LHCII (Rabhi et al., 2010). Moreover, the increase in Chl *a*/*b* ratio together with a parallel increase in VAZ/Chl *a* ratio (data not shown) and DEPS index may indicate an enrichment in pigment-protein complexes characterized by a relatively high Chl *a*/*b* ratio, as LHCI polypeptides and the PSII minor light harvesting proteins, CP29 and CP26 (Bassi et al., 1997; Jansson, 1994) which are believed to bind most of the xanthophylls cycle pigments (Bassi et al., 1993).

Differently from our results, Ramani et al. (2006) noticed no change in carotenoid content of *S. portulacastrum* cultivated under saline conditions. Such a different trend of response to salt stress may depend on the length of salt treatment performed

in the two experiments, which lasted 10 days in the research performed by Ramani et al. (2006), while in the present experiment it was prolonged for 13 weeks. In both species, the increased β-carotene content observed in salt-grown plants (Table 2) may result in increased antioxidant activity to protect photosystems against photooxidation. Koyro (2006) also observed an increase in the carotenoid/chlorophyll ratio and in leaf area ratio in the potential cash crop halophyte *Plantago coronopus* (L.). Actually, when absorbed light exceeds plant photochemical requirement (as may occur under normal light irradiation but in the presence of environmental constraints) this excess energy may be transferred to the ever-present oxygen. In this context, the conversion of violaxanthin to zeaxanthin through the xanthophyll cycle is considered to be one of the most effective energy dissipation mechanisms (Demmig-Adams and Adams, 1992). In *S. portulacastrum*, the lowest DEPS index was recorded at 200 mM NaCl, the salt concentration at which this species showed the highest CO_2 assimilation rate (Fig. 2). A similar result was observed also following a shorter period of saline growth (3 or 5 weeks) at both 200 and 400 mM NaCl (Rabhi et al., 2010). However, when salt treatment was extended to 13 weeks, plants grown at 400 mM NaCl exhibited a higher DEPS index (Table 2), indicating the need to alleviate excessive excitation pressure under a prolonged exposure to high salt concentration.

Salt-treated plants of *T. indica*, that showed a progressively-reduced photochemistry with increasing salinity, should be expected to activate the xanthophyll cycle. On the contrary, the lowest DEPS index values were detected under salinity. Although the results of this experiment do not allow establishment of the reasons for this behavior, it can be hypothesized that the presence of NaCl somehow inhibited the xanthophyll cycle and/or that under these salt concentrations the excitation pressure at the photosystem level is very low thanks to alternative energy dissipation mechanisms, as the water-water cycle (Asada, 2000) or a re-organization of the photosynthetic apparatus.

Acknowledgements

This research was supported by the Tunisian Ministry of Higher Education and Scientific Research (LR10CBBC02), by funds of the University of Pisa and by the European Cooperation in the field of Scientific and Technical Research, COST Action FA0901: “Putting Halophytes to Work — From Genes to Ecosystems”. We are also grateful to Professor Alberto Pardossi, Department of Crop Biology University of Pisa, Italy, for his kind help by providing us with a greenhouse for plant culture.

References

Abdelly, C., Lachâal, M., Grignon, C., Soltani, A., Hajji, M., 1995. Episodic association of strict halophytes and glycophytes in a hydromorphic saline ecosystem in semi-arid zone. Agronomie 15, 557–568.

Akhani, H., Trimborn, P., Ziegler, H., 1997. Photosynthetic pathways in *Chenopodiaceae* from Africa, Asia and Europe with their ecological, phytogeographical and taxonomical importance. Plant Systematics and Evolution 206, 187–221.

Asada, K., 2000. The water–water cycle as alternative photon and electron sinks. Philosophical Transaction of the Royal Society B: Biological Sciences 355, 1419–1431.

Bassi, R., Pineau, B., Dainese, P., Marquardt, J., 1993. Carotenoid-binding proteins of photosystem II. European Journal of Biochemistry 212, 297–303.

Bassi, R., Sandonà, D., Croce, R., 1997. Novel aspects of chlorophyll *a*/*b*-binding proteins. Physiologia Plantarum 100, 769–779.

Brugnoli, E., Björkman, O., 1992. Growth of cotton under continuous salinity stress: influence on allocation pattern, stomatal and non-stomatal components of photosynthesis and dissipation of excess light energy. Planta 187, 335–347.

Castagna, A., Nali, C., Ciompi, S., Lorenzini, G., Soldatini, G.F., Ranieri, A., 2001. Ozone exposure affects photosynthesis of pumpkin (*Cucurbita pepo*) plants. New Phytologist 152, 223–229.

Chaves, M.M., Flexas, J., Pinheiro, C., 2009. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Annals of Botany 103, 551–560.

Debez, A., Saadaoui, D., Ramani, B., Ouerghi, Z., Koyro, H.W., Huchzermeyer, B., Abdelly, C., 2006. Leaf H^+-ATPase activity and photosynthetic capacity of *Cakile maritima* under increasing salinity. Environmental and Experimental Botany 57, 285–295.

Demmig-Adams, B., Adams, W.W., 1992. Photoprotection and other responses of plants to high light stress. Annual Review of Plant Physiology and Plant Molecular Biology 43, 599–626.

Downton, W.J.S., Loveys, B.R., Grant, W.J.R., 1990. Salinity effects on the stomatal behaviour of grapevine. New Phytologist 116, 499–503.

Drake, B.G., 1989. Photosynthesis of salt marsh species. Aquatic Botany 34, 167–180.

Drew, M.C., Hole, P.C., Picchioni, G.A., 1990. Inhibition by NaCl of net CO_2 fixation and yield of cucumber. Journal of the American Society for Horticultural Science 115, 472–477.

Ehleringer, J.R., Cerling, T.E., Helliker, B.R., 1997. C_4 photosynthesis, atmospheric CO_2, and climate. Oecologia 112, 285–299.

Everard, J.D., Gucci, R., Kahn, S.C., Flore, J.A., Loescher, W.H., 1994. Gas exchange and carbon partitioning in the leaves of celery (*Apium graveolens* L.) at various levels of root zone salinity. Plant Physiology 106, 281–292.

Flowers, T.J., Colmer, T.D., 2008. Salinity tolerance in halophytes. New Phytologist 179, 945–963.

Ghnaya, T., Nouairi, I., Slama, I., Messedi, D., Grignon, C., Abdelly, C., Ghorbel, M.H., 2005. Cadmium effects on growth and mineral nutrition of two halophytes: *Sesuvium portulacastrum* and *Mesembryanthemum crystallinum*. Journal of Plant Physiology 162, 1133–1140.

Gibberd, M.R., Turner, M.C., Storey, R., 2002. Influence of saline irrigation on growth, ion accumulation and partitioning and leaf gas exchange of carrot (*Daucus carota* L.). Annals of Botany 90, 715–724.

Goldstein, G., Drake, D.R., Alpha, C., Melcher, P., Heraux, J., Azocar, A., 1996. Growth and photosynthetic responses of *Scaevola sericea*, a Hawaiian coastal shrub, to substrate salinity and salt spray. International Journal of Plant Sciences 157, 171–179.

Henderson, S.A., Von Caemmerer, S., Farquhar, G.D., 1992. Short-term measurements of carbon isotope discrimination in several C_4 species. Australian Journal of Plant Physiology 19, 263–285.

Hewitt, E.J., 1966. Sand and water culture methods used in the study of plant nutrition. Commonwealth Bureau of Horticulture and Plantation Crops. Technical Communication 22, 431–446.

Hunt, R., 1990. Basic Growth Analysis: Plant Growth Analysis for Beginners. Unwin Hyman, London.

Jansson, S., 1994. The light-harvesting chlorophyll *a*/*b*-binding proteins. Biochimica et Biophysica Acta 1184, 1–19.

Koyro, H.W., 2006. Effect of salinity on growth, photosynthesis, water relations and solute composition of the potential cash crop halophyte *Plantago coronopus* (L.). Environmental and Experimental Botany 56, 136–146.

Krishna Raj, S., Mawson, B.T., Yeung, E.C., Thorpe, T.A., 1993. Utilization of induction and quenching kinetics of chlorophyll a fluorescence for in vivo salinity screening studies in wheat (*Triticum aestivum* vars. Kharchia-65 and Fielder). Canadian Journal of Botany 71, 87–92.

Kubásek, J., Šetlík, J., Dwyer, S., Šantrůček, J., 2007. Light and growth temperature alter carbon isotope discrimination and estimated bundle sheath leakiness in C_4 grasses and dicots. Photosynthesis Research 91, 47–58.

Kurban, H., Saneoka, H., Nehira, K., Adilla, R., Premachandra, G.S., Fujita, K., 1999. Effect of salinity on growth, photosynthesis and mineral composition in leguminous plant *Alhagi pseudoalhagi* (Bieb.). Soil Science and Plant Nutrition 45, 851–862.

Lakshmi, A., Ramanjulu, S., Veeranjaneyulu, K., Sudhakar, C., 1996. Effect of NaCl on photosynthesis parameters in two cultivars of mulberry. Photosynthetica 32, 285–289.

Le Houérou, H.N., Ionesco, T., 1973. Palatability of plant species from steppic Tunisia. AG-TUN 71/ 525 FAO, Rome.

Lieth, H., Moschenko, M., Lohmann, M., Koyro, H.W., Hamdy, A., 1999. Halophyte uses in different climates. I. Ecological and ecophysiological studies. Progress in Biometeorology. Backhuys Publishers, Leiden.

Locy, R.D., Chang, C.C., Nielsen, B.L., Singh, N.K., 1996. Photosynthesis in salt-adapted heterotrophic tobacco cells and regenerated plants. Plant Physiology 110, 321–328.

Lokhande, V.H., Nikam, T.D., Suprasanna, P., 2009. *Sesuvium portulacastrum* (L.) L. a promising halophyte: cultivation, utilization and distribution in India. Genetic Resources and Crop Evolution 56, 741–747.

Lonard, R.I., Judd, F.W., 1997. The biological flora of coastal dunes and wetlands. *Sesuvium portulacastrum* (L.) L. Journal of Coastal Research 13, 96–104.

Magwa, M.L., Gundidza, M., Gweru, N., Humphrey, G., 2006. Chemical composition and biological activities of essential oil from the leaves of *Sesuvium portulacastrum*. Journal of Ethnopharmacology 103, 85–89.

Malibari, A.A., Zidan, M.A., Heikal, M.M., El-Shamary, S., 1993. Effect of salinity on germination and growth of alfalfa, sunflower and sorghum. Pakistan Journal of Botany 25, 156–160.

Messedi, D., Sleimi, N., Abdelly, C., 2003. Some physiological and biochemical aspects of salt tolerance of *Sesuvium portulacastrum*. In: Lieth, H., Moschenko, M. (Eds.), Cash Crop Halophytes: Recent Studies. Kluwer Academic Publishers, Dordrecht, pp. 71–77.

Morgan, J.A., LeCain, D.R., Pendall, E., Blumenthal, D.M., Kimball, B.A., Carrillo, Y., Williams, D.G., Heisler-White, J., Dijkstra, F.A., West, M., 2011. C_4 grasses prosper as carbon dioxide eliminates desiccation in warmed semi-arid grassland. Nature 476, 202–205.

Munns, R., 2002. Comparative physiology of salt and water stress. Plant, Cell and Environment 25, 239–250.

Munns, R., Greenway, H., Delane, R., Gibbs, J., 1982. Ion concentration and carbohydrate status of the elongating leaf tissue of *Hordeum vulgare* growing at high external NaCl: II Cause of the growth reduction. Journal of Experimental Botany 33, 574–583.

Murchie, E.H., Pinto, M., Horton, P., 2009. Agriculture and the new challenges for photosynthesis research. New Phytologist 181, 532–552.

Nagarajan, D., Sivasankaramoorthy, S., Venkatesan, A., 2008. Salinity tolerance on growth and organic content of *Arthrocnemum indicum* Moq. Plant Archives 8, 245–248.

Nieva, F.J.J., Castellanos, E.M., Figueroa, M.E., Gil, F., 1999. Gas exchange and chlorophyll fluorescence of C_3 and C_4 saltmarsh species. Photosynthetica 36, 397–406.

Osborne, C.P., Wythe, E.J., Ibrahim, D.G., Gilbert, M.E., Ripley, B.S., 2008. Low temperature effects on leaf physiology and survivorship in the C_3 and C_4 subspecies of *Alloteropsis semialata*. Journal of Experimental Botany 59, 1743–1754.

Parida, A.K., Das, A.B., Mittra, B., 2004. Effects of salt on growth, ion accumulation photosynthesis and leaf anatomy of the mangrove, *Bruguiera parviflora*. Trees: Structure and Function 18, 167–174.

Rabhi, M., Hafsi, C., Lakhdar, A., Hajji, S., Barhoumi, Z., Hamrouni, M.H., Abdelly, C., Smaoui, A., 2009. Evaluation of the capacity of three halophytes to desalinize their rhizosphere as grown on saline soils under nonleaching conditions. African Journal of Ecology 47, 463–468.

Rabhi, M., Giuntini, D., Castagna, A., Remorini, D., Baldan, B., Smaoui, A., Abdelly, C., Ranieri, A., 2010. *Sesuvium portulacastrum* maintains adequate gas exchange, pigment composition, and thylakoid proteins under moderate and high salinity. Journal of Plant Physiology 167, 1336–1341.

Rajesh, A., Arumugam, R., Venkatesalu, V., 1998. Growth and photosynthetic characteristics of *Ceriops roxburghiana* under NaCl stress. Photosynthetica 35, 285–287.

Ramani, B., Reeck, T., Debez, A., Stelzer, R., Huchzermeyer, B., Schmidt, A., Papenbrock, J., 2006. *Aster tripolium* L. and *Sesuvium portulacastrum* L.: two halophytes, two strategies to survive in saline habitats. Plant Physiology and Biochemistry 44, 395–408.

Ravindran, K.C., Venkatesan, K., Balakrishnan, V., Chellappan, K.P., Balasubramanian, T., 2007. Restoration of saline land by halophytes for Indian soils. Soil Biology and Biochemistry 39, 2661–2664.

Sage, R.F., 2004. The evolution of C_4 photosynthesis. New Phytologist 161, 341–370.

Solomon, A., Beer, S., Waisel, Y., Jones, G.P., Paleg, L.G., 1994. Effects of NaCl on the carboxylation activity of Rubisco from *Tamarix jordanis* in the presence and absence of proline-related compatible solutes. Physiologia Plantarum 90, 198–204.

Sudhir, P., Murthy, S.D.S., 2004. Effects of salt stress on basic processes of photosynthesis. Photosynthetica 42, 481–486.

Tazoe, Y., Hanba, Y.T., Furumoto, T., Noguchi, K., Terashima, I., 2008. Relationships between quantum yield for CO_2 assimilation, activity of key enzymes and CO_2 leakiness in *Amaranthus cruentus*, a C_4 dicot, grown in high or low light. Plant & Cell Physiology 49, 19–29.

Venkatesalu, V., Chellappan, K.P., 1993. Photosynthetic characteristics of *Sesuvium portulacastrum* L. under salt stress. Photosynthetica 28, 313–316.

Voznesenskaya, E.V., Akhani, H., Koteyeva, N.K., Chuong, S.D.X., Roalson, E.H., Kiirats, O., Franceschi, V.R., Edwards, G.E., 2008. Structural, biochemical, and physiological characterization of photosynthesis in two C_4 subspecies of *Tecticornia indica* and the C_3 species *Tecticornia pergranulata* (Chenopodiaceae). Journal of Experimental Botany 59, 1715–1734.

Weber, D.J., Ansari, R., Gul, B., Khan, M.A., 2007. Potential of halophytes as source of edible oil. Journal of Arid Environments 68, 315–321.

Yeo, A.R., Flowers, F.J., 1980. Salt tolerance in the halophyte Suaeda maritima L. Dum.: Evaluation of the effect of salinity upon growth. Journal of Experimental Botany 31, 1171–1183.

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