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Photosynthesis and photosystem 2 efficiency of two salt-adapted halophytic seashore *Cakile maritima* ecotypes

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Abstract

The effects of salinity (0–400 mM NaCl, marked S_0, S_{100}, S_{200}, and S_{400}) on growth, photosynthesis, photosystem 2 (PS2) efficiency, ion relations, and pigment contents were studied in two seashore *Cakile maritima* ecotypes (Tabarka and Jerba, respectively, sampled from humid and arid bioclimatic areas). Growth of Jerba plants was improved at S_{100} as compared to S_0. Tabarka growth was inhibited by salinity at all NaCl concentrations. Leaf sodium and chloride concentrations increased with medium salinity and were higher in Jerba than in Tabarka plants. Chlorophyll content, net photosynthetic rate, stomatal conductance (g_s), and intracellular CO_2 concentration were stimulated at moderate salinity (S_{100}) in Jerba plants and inhibited at higher salt concentrations in both ecotypes: g_s was the most reduced parameter. The maximum quantum efficiency of PS2 (F_v/F_m), quantum yield, linear electron transport rate, and efficiency of excitation energy capture by open PS2 reaction centres showed no significant changes with increasing salt concentration in Jerba plant and were decreased in Tabarka subjected to S_{400}. However, the efficiency of dissipation of excess photon energy in the PS2 antenna was maintained in Jerba and was increased in Tabarka plants challenged with S_{400}. Hence the relative salt tolerance of Jerba was associated with a better ability to use Na^+ and Cl^- for osmotic adjustment, the absence of pigment degradation, and the concomitant PS2 protection from photodamage.

Additional key words: carotenoids; chlorophylls; fluorescence; intracellular CO_2 concentration; quenching; relative growth rate; salt tolerance; stomatal conductance; transpiration rate; water use efficiency.

Introduction

Salt stress depresses greatly growth and crop productivity, and soil salinity is one of major environmental constraints on agriculture in many regions of the world (Jaleel *et al.* 2007c). In the arid and semi-arid zones, the combination of inappropriate irrigation practices and high evaporative-transpiration rates are largely responsible for extending soil/water salinization (Owens 2001, Debez *et al.* 2006). Limitation of plant growth by salinity cannot be assigned to a single physiological process. Photosynthesis is an important parameter used to monitor plant response to abiotic constraints (Zhao *et al.* 2007). A decline in photosynthetic capacity often occurs in many plants when subjected to salinity stress (Qiu *et al.* 2003). Salinity changes photosynthetic parameters, including osmotic and leaf water potential, transpiration rate, leaf temperature, and relative leaf water content (Munns 2002, Zhao *et al.* 2007). Salt also affects contents of chlorophyll (Chls) and carotenoids (Car), and reduces efficiency of ribulose-1,5-bisphosphate carboxylase/oxygenase for carbon fixation (Delfine *et al.* 1998, Lee *et al.* 2004, Jaleel *et al.* 2007a). Changes in these parameters depend on the severity and duration of stress (Lakshmi *et al.* 1996, Misra *et al.* 1997, Jaleel *et al.* 2007b) and on plant species (Dubey 1994). A consequence of the salinity-induced limitation of photosynthetic capacity is the exposure of plants to excess energy, which, if not safely dissipated, may be harmful to PS2 due to over-reduction of reaction centres (Demmig-Adams and Adams 1996).

One of the mechanisms by which plants avoid photodamage is radiation-less dissipation of the excess excitation energy by the xanthophyll cycle observed under various environmental stresses (Qui *et al.* 2003). Car (carotenes and xanthophylls), besides acting as accessory light-harvesting pigments, show antioxidant properties

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(de Pascale *et al*. 2001), are essential components of the light-harvesting complex, and can also quench the exited triplet state of Chl, thus indirectly reducing the formation of singlet. The less reduction in Chl and steady contents of Car of sugarcane are salinity tolerance strategies (Kanhaiya 1996), and the steady contents of Car are tightly correlated with salt tolerance in mungbean (Wahid *et al*. 2004).

Plants under stress utilize less photon energy for photosynthesis. Some of the excessive energy is quenched into Chl fluorescence to minimize damage to photosynthetic systems, particularly in photosystem 2 (PS2) and subsequent electron carriers (Krause and Weis 1991, Wang *et al*. 1999). Salinity stress can predispose plants to photoinhibition and photodamage of PS2 (Belkhodja *et al.* 1994, Qiu *et al*. 2003). Measurements of Chl fluorescence, like effective quantum yield of PS2 (F_v'/F_m'), and the estimation of the electron transport rate (ETR) make it possible to evaluate the plant's photosynthetic performance and the extent of its tolerance to environmental stress (Maxwell and Johnson 2000, Broetto *et al*. 2007).

Cakile maritima (Brassicaceae) is an annual C_3 fleshy-leaf oilseed halophyte, which strictly colonizes the sandy beaches, thus contributing to dune formation and stabilization (Hocking 1982). This species, very frequent in Tunisia from the north (humid climate) to the sub-Sahara region, shows considerable variability, within and between subspecies (Davy *et al*. 2006). In previous studies (Ben Amor *et al.* 2006, Megdiche *et al*. 2007) we showed that the salt response of this species was both accession and development stage dependent. Objectives of the present study were: (*a*) to evaluate differences in photosynthetic responses of two *C. maritima* ecotypes that vary in salinity tolerance; (*b*) to establish the characteristics of photosynthetic parameters associated with salinity tolerance; and (*c*) to assess the potential use of Chl and Car contents and Chl fluorescence as screening indexes for salinity tolerance in halophytic *C. maritima* plants.

Materials and methods

Plants and growth conditions: Seeds of two Tunisian seashore *C. maritima* were collected from Tabarka (humid bioclimatic area, longitude (L)/latitude (l): 8°45'E/36°57'N) and Jerba (south, arid bioclimatic area, 11°E/33°50'N, respectively), which show difference in salt tolerance, were used (cf. Megdiche *et al.* 2007). Seeds were germinated in pots filled with inert and humid sand. Ten-day-old seedlings, selected for their uniformity in size and form, were cultivated in pots under greenhouse conditions (25±5 °C temperature, 60±10 % relative humidity). Three-week-old plants were then irrigated at alternate days with a nutrient solution (Hewitt 1966) at pH 7.3, containing 0, 100, 200, or 400 mM NaCl (S_0, S_{100}, S_{200}, S_{400}). Twenty days after salt addition to the culture medium, leaf samples (taken from 4 plants per treatment) were used for monitoring the main parameters.

Growth and water relations: Dry mass (DM) of the shoot samples was estimated after their drying at 60 °C for 72 h. Plant relative growth rate (RGR) was determined as: $RGR = \Delta M/\underline{M}\ \Delta t$, where Δ is the difference between values at the final and initial harvests, t is the time [d], and M is the whole plant DM [g]. $\underline{M}$ is the logarithmic mean of M calculated over the Δt period (Hunt 1990): $\underline{M} = \Delta M/\Delta \ln(M)$. The net assimilation rate (NAR) was determined by dividing the leaf mass fraction (LMF) by its specific leaf area (SLA) between values at the final (t_2) and initial harvests (t_1) (Abdelly *et al.* 1995) and was calculated as: $NAR = (DM_2 - DM_1)/SLA\ (t_2 - t_1)$, with $SLA = (LA_2 - LA_1)/\ln(LA_2/LA_1)$. Total leaf area (LA) was measured by a leaf area meter (portable area meter *LI/3000A*, *LI-COR*). Relative water content (RWC) was measured in the 2nd or 3rd youngest fully expanded leaf harvested in the morning. These two parameters were determined using the following equation (Schönfeld *et al.* 1988): RWC [%] = 100 (FM – DM)/(TM – DM). FM (fresh mass) was determined after harvest. Turgid mass (TM) was obtained after soaking leaves in distilled water in test tubes for 12 h at room temperature (about 20 °C), under low irradiance of laboratory. After soaking, leaves were quickly and carefully blotted dry with tissue paper in preparation for determining TM. DM was obtained after oven drying leaf samples at 60 °C for 72 h.

Determination of sodium and chloride: After ion extraction from leaf samples [30 mg(DM) each] in 0.5 % HNO_3, chloride was assayed by coulometry (Büchler chloridometer) and Na^+ by flame emission photometry (*Corning*, UK).

Pigment analyses: Chl and Car contents in mature leaves were determined according to Torrecillas *et al.* (1984) and Arnon (1949), respectively. Five millilitres of pure acetone were added to fresh leaf samples cut into discs (*ca.* 100 mg each). The extraction took place in darkness at 4 °C for 72 h. Chl contents were measured at 649 and 665 nm, Car content at 470, 663, and 647 nm.

Gas exchange: Net photosynthetic rate (P_N), stomatal conductance (g_s), intracellular CO_2 concentration (C_i), and transpiration rate (E) were determined by using a portable photosynthesis system (*LCA4*). Measurements were carried out between 10:00 and 12:00 on leaves (10 replicates per treatment). Data were automatically collected every minute after photosynthesis rate was stabilized.

Chl fluorescence emission from the upper surface of the leaves of intact plants was measured by modulated fluorimeter (*MINI-PAM*; *Walz*, Effeltrich, Germany). After dark-adaptation of samples for 1 h, the minimum fluorescence (F_0) in the dark-adapted state (DAS) was measured with weak modulated irradiation (<0.1 µmol m^{-2} s^{-1}). A 600-ms saturating flash (>7 000 µmol m^{-2} s^{-1}) was applied to determine the maximum Chl fluorescence yield in the DAS (F_m) and the maximum quantum efficiency of PS2 photochemistry $F_v/F_m = (F_m - F_0)/F_m$ (Baker and Rosenqvist 2004). Then the leaves were continuously irradiated with "white actinic light", which was equivalent to the actual growth irradiance of *C. maritima* plants in order to measure F_s and F_m' (steady-state and maximum fluorescence yield in irradiated leaves, LAS, respectively). F_0' (minimum fluorescence in the LAS) was estimated following Baker and Rosenqvist (2004). Non-photochemical quenching of fluorescence (NPQ), proportional to the rate constant of thermal energy dissipation, was calculated as $F_m - F_m$')/F_m' (Björkman and Demmig-Adams 1994). The coefficient of photochemical quenching (q_p) was calculated as (F_m' – F_s)/(F_m' – F_0') (Schreiber *et al.* 1986, Van Kooten and Snel 1990). The intrinsic efficiency of open PS2, Φ_{exc} (or excitation capture efficiency of PS2 units) was calculated as F_v'/F_m'. The Φ_{PS2} (or actual PS2 efficiency) was calculated as F_q'/F_m' = (F_m' – F_s)/F_m' (Genty *et al.* 1989, Schreiber *et al.* 1995). Φ_{PS2} was used for calculation of the linear ETR according to Krall and Edwards (1992) as ETR = $\Delta F/F_m$'×PPFD×0.5×0.84, where PPFD is photosynthetic photon flux density incident on the leaf; 0.5 is factor that assumes equal distribution of energy between the two photosystems; and 0.84 is assumed leaf absorbance. The efficiency of dissipation of excess photon energy in the PS2 antenna (D) was calculated as $1 - \Phi_{exc} = 1 - (F_v$'/$F_m$') (Demmig-Adams *et al.* 1996).

Statistical analysis: A two-way analysis of variance (ANOVA) with the ecotype and treatment as factors, and their interaction was performed for the whole data set using the *STATI-CF* statistical program. Means were compared using the Newman-Keuls test at the $p<0.05$ level when significant differences were found. Correlation analysis was carried out using the correlation and regression program in the *EXCEL* program.

Results

Plant growth, leaf water status, and salt accumulation: The effect of salinity (S), ecotype (E) and their interaction (S×E) on these parameters is in Table 1. Moderate NaCl concentration (S_{100} supplied during 20 d) stimulated transiently the RGR only in Jerba ecotype (*ca.* 115 % of the control), which seems to be optimal for the growth of this ecotype. At S_{200}, RGR was *ca.* 87 and 71 % of the control in Jerba and Tabarka, respectively (Fig. 1*A*). S_{400} inhibited RGR by 28 and 52 % in Jerba and Tabarka, respectively.

In Jerba plants, NAR was significantly enhanced at S_{100} (*ca.* 125 % of the control; Fig. 1*B*). Even at S_{200} and S_{400}, NAR remained higher in this ecotype (*ca.* 92 and 88 % of the control, respectively). In the contrary, this parameter was significantly affected at all salt treatments in Tabarka. However, there was no change in leaf relative water content in Jerba and Tabarka at moderate salinity (Fig. 2*A*). Up to S_{200}, this parameter significantly increased in Jerba and decreased in Tabarka. Leaf sodium and chloride contents increased with medium salinity and were higher in Jerba than in Tabarka (Fig. 2*B*).

Pigment contents and gas exchange: When grown at S_{100}, Jerba showed an enhancement of Chl contents, while a slight decline was observed in Tabarka (82 % of the control; Fig. 3*A*). At higher salinities, the reduction of Chl (*a*+*b*) and Chl *b* did not exceed 31 % in the two ecotypes. In contrast, content of Chl *a* was not affected significantly by salinity. Car contents were significantly different among the ecotypes and salt concentrations. Under salt stress, Jerba maintained a steady content of Car whereas Tabarka showed a steep decline (up 42 % at S_{400}; Fig. 3*B*). Indeed, salinity significantly increased

Table 1. Two-way analysis of variance of plant characteristics by salinity (S), ecotype (E), and their interaction (S×E). Numbers represent *F* values: $^{*}p<0.01$, $^{**}p<0.001$, $^{***}p<0.0001$.

Dependent variable	S	E	S×E
RGR	197.13^{***}	0.86	27.68^{***}
NAR	2782.96^{***}	830.59^{***}	1070.87^{***}
RWC	2.85^{*}	53.14^{***}	54.89^{***}
Na^+	2225.47^{***}	686.90^{***}	214.63^{***}
Cl^-	173.91^{***}	7.50^{*}	9.92^{***}
Chl *a*	49.28^{***}	8.59^{**}	3.21^{*}
Chl *b*	12.39^{***}	2.24	5.94^{**}
Chl (*a*+*b*)	67.66^{***}	11.99^{**}	8.10^{**}
Car	19.30^{***}	86.23^{***}	21.32^{***}
Car/Chl	23.52^{***}	1121.56^{***}	47.13^{***}
P_N	380.61^{***}	295.17^{***}	21.86^{***}
g_s	1801.62^{***}	549.74^{***}	91.18^{***}
E	142.67^{***}	91.22^{***}	16.65^{***}
*C*i	174.71^{***}	158.33^{***}	39.35^{***}
WUE	32.17^{***}	31.58^{***}	32.58^{***}
F_0	4.12^{**}	17.95^{***}	2.75^{*}
F_m	1.74	1.10	4.61^{**}
F_v/F_m	3.22^{*}	6.27^{*}	16.30^{***}
Φ_{PS2}	3.86^{*}	0.05	6.00^{**}
Φ_{exc}	5.90^{**}	0.75	2.79^{**}
q_p	2.75^{*}	0.69	0.17
NPQ	48.58^{***}	52.15^{***}	14.57^{***}
D	11.08^{***}	2.44	14.57^{***}
ETR	6.78^{***}	1.47	3.02^{*}

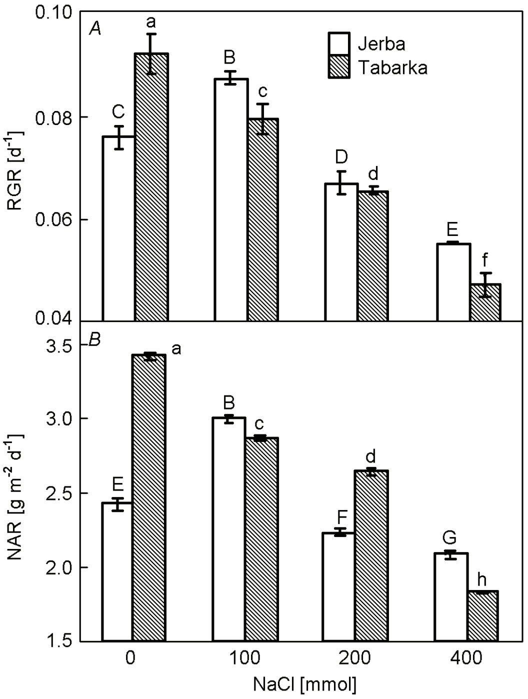

Fig. 1. Effect of increasing NaCl concentrations on relative growth rate (*A*) and net assimilation rate (*B*) in leaves of Jerba and Tabarka ecotypes (*n* = 4 ± SE) of *Cakile maritima*. Values followed by at least one same letter were not significantly different at $p<0.05$ according to the Newman-Keuls post-hoc test.

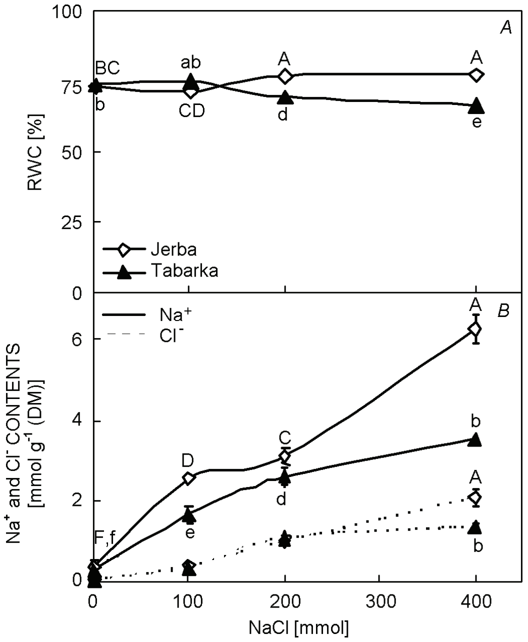

Fig. 2. Effect of increasing NaCl concentrations on relative water content (*A*) and Na^+ and Cl^- concentrations (*B*) in leaves of Jerba and Tabarka ecotypes (*n* = 4 ± SE) of *Cakile maritima*. Values followed by at least one same letter were not significantly different at $p<0.05$ according to the Newman-Keuls post-hoc test.

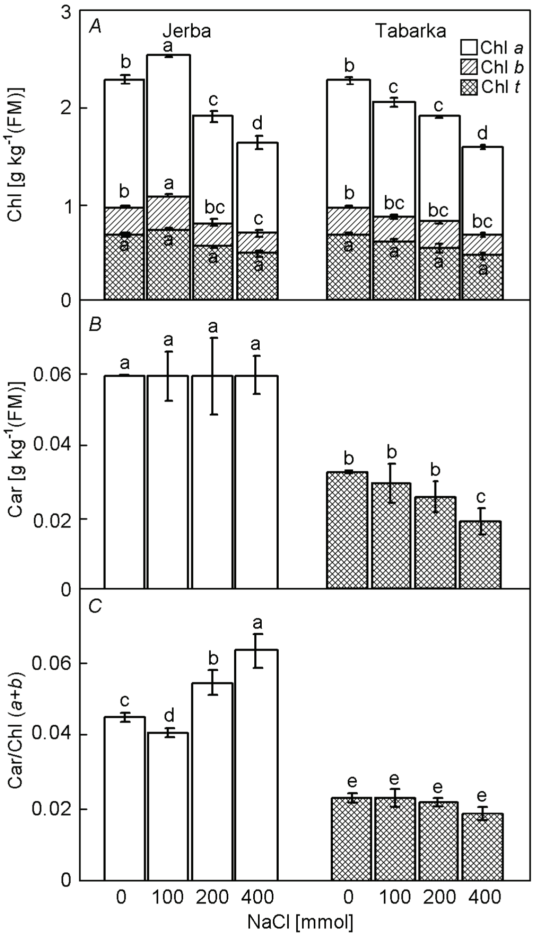

Fig. 3. Effect of increasing NaCl concentrations on contents of chlorophylls (*A*) and carotenoids (*B*), and on Car/Chl ratio in leaves of Jerba and Tabarka ecotypes (*n* = 4 ± SE) of *Cakile maritima*. Values followed by at least one same letter were not significantly different at $p<0.05$ according to the Newman-Keuls post-hoc test.

the Car/Chl ratio in Jerba, and this ratio was maintained fairly steady (just a 15.6 % decrease) under higher salinity in Tabarka (Fig. 3*C*).

Under moderate salinity stress, P_N was enhanced significantly in Jerba and decreased in Tabarka (Fig. 4*A*). Higher salt concentrations strongly reduced P_N (*ca.* 53 and 77 % lower than control at S_{400} in Jerba and Tabarka, respectively). The g_s, *E*, and C_i displayed a similar pattern, though C_i was much less affected by salt (Fig. 4*B–D*). At S_{200}, C_i decreased by 5 % for Jerba and by 21 % for Tabarka. At S_{400}, it was reduced by 23 and 33 % in Jerba and Tabarka, respectively. At S_{100}, WUE increased in Tabarka (Fig. 4*E*). At S_{400}, WUE was not affected in both Jerba and Tabarka ecotypes as compared to their respective control, owing to the considerable decrease in leaf *E* following the salt-induced stomatal closure.

Table 2. Effect of different salt treatments on maximum quantum efficiency of PS2 (F_v/F_m), quantum yield of PS2 electron transport (Φ_{PS2}), intrinsic efficiency of open PS2 (Φ_{exc}), photochemical quenching (q_p), non-photochemical quenching (NPQ), efficiency of dissipation of excess photon energy in the PS2 antenna (D), and linear electron transport rate (ETR) in leaves of Jerba and Tabarka ecotypes (*n* = 10). Values followed by at least one same letter were not significantly different at $p<0.05$, according to the Newman-Keuls post-hoc test.

		S_0	S_{100}	S_{200}	S_{400}
F_0	Jerba	157.80±6.11a	154.00±7.88a	148.22±6.78ab	152.11±9.54ab
	Tabarka	142.92±5.63b	145.80±7.19ab	152.10±6.98ab	132.80±3.65c
F_m	Jerba	923.80±31.43b	1007.40±25.84a	952.30±30.00ab	943.33±52.71ab
	Tabarka	980.61±37.79ab	956.60±40.79ab	936.70±15.52ab	984.70±42.43ab
F_v/F_m	Jerba	0.835±0.003d	0.849±0.004b	0.848±0.002b	0.848±0.003bc
	Tabarka	0.856±0.003a	0.848±0.006bc	0.853±0.003b	0.843±0.002cd
Φ_{PS2}	Jerba	0.786±0.009ab	0.782±0.020ab	0.800±0.006a	0.794±0.008a
	Tabarka	0.801±0.006a	0.791±0.008a	0.797±0.005a	0.774±0.006b
Φ_{exc}	Jerba	0.833±0.008a	0.837±0.010a	0.839±0.006a	0.828±0.013a
	Tabarka	0.844±0.019a	0.839±0.010a	0.833±0.010a	0.808±0.020b
q_p	Jerba	0.944±0.008a	0.933±0.024a	0.954±0.012a	0.960±0.018a
	Tabarka	0.951±0.025a	0.944±0.017a	0.957±0.009a	0.959±0.012a
NPQ	Jerba	0.020±0.004d	0.047±0.011d	0.053±0.012d	0.142±0.021c
	Tabarka	0.029±0.010d	0.055±0.011d	0.210±0.015b	0.281±0.024a
D	Jerba	0.167±0.008c	0.163±0.009a	0.161±0.006c	0.172±0.004c
	Tabarka	0.156±0.006c	0.161±0.006c	0.167±0.005c	0.192±0.003b
ETR	Jerba	524.712±5.207a	529.946±5.095a	528.281±3.986a	521.865±8.214a
	Tabarka	531.770±7.220a	528.605±6.403a	524.537±3.292a	508.734±6.896b

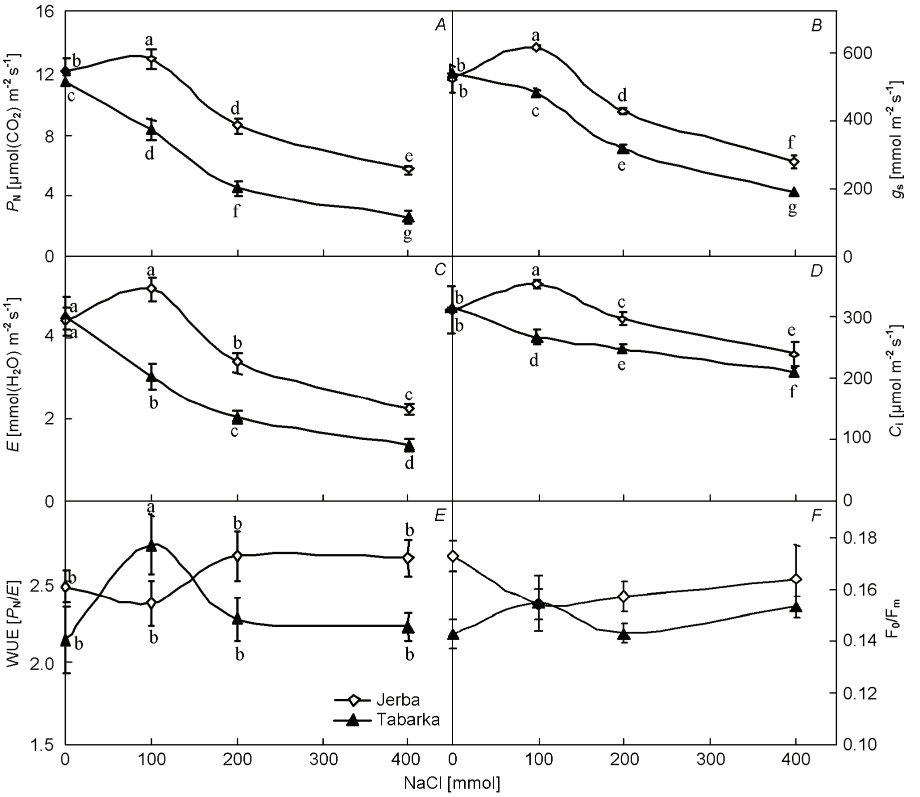

Fig. 4. Effect of increasing NaCl concentrations on (*A*) net photosynthetic rate, P_N; (*B*) stomatal conductance, g_s; (*C*) transpiration rate, *E*; (*D*) internal CO_2 concentration, C_i; (*E*) water use efficiency, WUE; and (*F*) F_0/F_m ratio in leaves of Jerba and Tabarka ecotypes (*n* = 10 ± SE). Values followed by at least one same letter were not significantly different at $p<0.05$, according to the Newman-Keuls post-hoc test.

Chl fluorescence: The values of maximal efficiency of PS2 photochemistry (F_v/F_m) in both control and salt-subjected plants were around 0.8 and exhibited a slight increase in Jerba plants subjected to S_{100}. No increase was recorded at higher salinities in this ecotype. In Tabarka plants, this parameter was not significantly modified by salt treatment. The initial fluorescence (F_0) was not affected by salt stress in Jerba, whereas it was significantly decreased at S_{400} in Tabarka. The F_m was similar in both ecotypes, but only in Jerba F_m was increased significantly at S_{100}. In light-adapted plants, there was no significant change in the quantum yield of PS2 electron transport (Φ_{PS2}) and in the efficiency of excitation energy capture by open PS2 reaction centres ($\Phi_{exc} = F_v'/F_m'$) with increasing salt concentration in Jerba, whereas these two parameters were decreased at 400 mM in Tabarka. However, there were no differences in F_v'/F_m' compared to F_v/F_m in Jerba and there was negligible decrease in Tabarka at S_{400} (*ca*. 4.2 %). The NPQ increased significantly at S_{400} in Jerba and up S_{200} in Tabarka. In plants challenged with S_{400}, NPQ in Jerba and Tabarka was *ca*. 7.1- and 9.6-fold more important than in the control, respectively (Table 2). There was no significant change in the photochemical quenching coefficient (q_p) in salt-adapted leaves. The linear ETR was no affected in Jerba ecotype and was significantly decreased at high salinity in Tabarka. The efficiency of dissipation of excess photon energy in the PS2 antenna (D) was maintained in Jerba (except at S_{100}) and was increased only in Tabarka plants challenged with S_{400} (Table 2).

Discussion

C. maritima is a halophyte adapted to growth in the coast regions with salinity. We found that its ability to cope with severe salt stress is a result of several mechanisms mainly involved in vacuole salt compartmentation and the conservation of the integrity of photosynthetic apparatus.

At S_{100}, the whole plant growth estimated by RGR and NAR (Table 1) was slightly decreased in the relatively susceptible ecotype Tabarka and significantly improved in the tolerant one, Jerba. Under severe salinity (up to S_{400}), the two ecotypes maintained also an important growth activity, though lower than that of the control plants without the appearance of toxicity symptoms (chlorosis and/or necrosis). Moreover, the leaf Na^+ and Cl^- concentrations were about 20 % of the dry matter (Fig. 2*B*) and reached the highest values in the ecotype which preserved best its growth activity. This large accumulation of salt in leaves, which was compatible with a high growth activity, can be explained only by a vacuolar salt compartmentation and its use for the osmotic adjustment. *C. maritima* may use the inclusive strategy when dealing with salinity. This assumption was supported by the increase of the leaf vacuolar V-H^+-ATPase activity up to 300 mM NaCl and the absence of salt excretion structures at the leaf surface (Debez *et al.* 2006). This property determines the conservation of water tissue status. Indeed, the values of RWC (Fig. 2*A*) were close to 70 % in plants subjected to salt and generally higher in the ecotype accumulating more salt in leaves. The conservation of the tissue hydration in plants subjected to salt is also the consequence of a regulation of the stomatal gas exchange. Indeed, we found that P_N was enhanced in Jerba and decreased in Tabarka at moderate salinity. Starting with S_{200}, there was a remarkable drop in P_N especially in the sensitive ecotype. The detrimental effect of salinity on P_N has been shown by Bañuls and Primomilo (1992) or Gebauer *et al*. (2004). The plant reacts to salt stress with a closure of stomata to avoid further loss of water through transpiration (Lee *et al.* 2004). As consequence, the diffusion of CO_2 into the leaf is restricted (Haupt-Herting and Fock 2000) and the growth is limited. The limitation in g_s and E can represent an adaptive mechanism to cope with excessive salt, rather than merely a negative consequence of it (Clark *et al.* 1999). Studies on *Plantago coronopus* and *Spartina townsendii* (Koyro 2003, 2006) showed that their threshold salinity tolerance was the combination of low (but positive) P_N, minimum E, high g_s, and minimum C_i. This strategy tends to reduce the salt loading into the leaves and helps increase the longevity by maintaining salt at below toxic levels longer than it would occur if E was not diminished (Everard *et al.* 1994). This regulation of the stomatal gaseous exchange in plants subjected to salt results in an economical use of water for growth. We state that this parameter does not undergo significant changes with increasing salinity in culture medium. However, an improvement of WUE has been reported in plants subjected to long-term effect of drought, salinity, or combined effect of these constraints (Slama *et al.* 2008). Indeed, the water losses by transpiration were lower in plants subjected to salt as consequence of high retention of water by leaves considering their richness in Na^+ and probably in other organic solutes.

Non-stomatal effects may also prevent photosynthetic activities as has been reported for several plants (Ranjbarfordoei *et al*. 2006, Rouhi *et al.* 2007) as well as the involvement of the photosynthetic pigments in protection against the photodamage (Qiu *et al.* 2003). Thus, the changes in photosynthetic pigment contents were analyzed in the two ecotypes of *C. maritima*. We found that salt led in *Cakile* to a steady state of Chl *a* and decrease in the Chl (*a*+*b*) and *b* contents. The changes in photosynthetic pigments depend on plant status, tolerant or sensitive. In crop plants relatively sensitive to salt like *Pistacia* (Ranjbarfordoei *et al.* 2002), rice (Singh and Dubey 1995), and *Sorghum bicolor* (Jagtap *et al.* 1998), Chl content decreased in response to an increasing salt stress. A decrease in contents of both Chl *a* and *b* of plants subjected to salt stress was attributed to destruction of Chl *a*, which is more sensitive than Chl *b*, and an increase in activity of degrading enzyme chlorophyllase which is more active under salt stress, whereas in halophyte species, like *Atriplex centralasiatica*, contents of Chl (*a*+*b*) were unchanged with increasing salt concentration (Qiu *et al.* 2003). This difference between halophyte and glycophyte species suggests that chloroplasts are subjected to the direct toxic effect of salt in the first and are protected from these effects in the second ones as consequence of a vacuolar compartmentation of salt. Additionally, under salinity the Car content in leaves of salt-tolerant plants was maintained and was decreased in Tabarka (Fig. 3*B*). Car dissipate excess energy in the photosystems 1 and 2 as heat or in non-damaging chemical reactions (Lu *et al*. 2003) and stabilize the chloroplast membranes (Havaux 1998). Car also protect the photosynthetic membrane from photo-oxidation by effectively scavenging singlet oxygen and the quenching triplet state of Chl (Adams *et al.* 1992). Under stress, an accumulation of excited molecules in the pigment bed, like the ^{3}Chl* and singlet oxygen, might occur (Foyer *et al.* 1994). These highly cytotoxic species and their deleterious products can seriously disrupt metabolism through oxidative damage to cellular components (De Vos *et al.* 1989). The minimal reduction in the contents of photosynthetic pigments is important, as it has a direct relationship with salinity tolerance (Ahmad *et al.* 2005). A supposed maintenance of Car in the tolerant ecotype and increased Car/Chl ratio revealed that, in addition to acting as accessory light-harvesting pigments, the Car provide a protective advantage against salt-induced oxidative damage.

When measured in DAS, there were no changes in the maximal efficiency of PS2 photochemistry (F_v/F_m) in Jerba salt-adapted leaves, suggesting no damage to PS2 (Table 2). In contrast, leaf Chl fluorescence responses decreased to salt stress in Tabarka plants which indicated that PS2 of Jerba plants was more tolerant to salinity. Moreover, when measured in LAS, although there was only a decrease in F_v'/F_m' at S_{400} in Tabarka, we observed that there were no significant differences in F_v'/F_m' between control and salt-adapted Jerba plants (Table 2). The fact that the decreased F_v'/F_m' in Jerba was completely recovered on dark condition and there were no important changes in F_0 indicated that photoinhibition observed in this ecotype was due to photo-protective processes and not to photo-inhibitory damage (Qui *et al.* 2003). These results indicate that the sensitivity of photoinhibition was not increased particularly in Jerba ecotype, even when subjected to salinity up to S_{400}.

The basal quantum yield of non-photochemical processes in PS2, F_0/F_m, covers the influences of both Chl fluorescence and non-radiative energy dissipation in DAS when all PS2 reaction centres are open (Roháček 2002). Based on the values of F_0/F_m (Fig. 4*F*), F_0/F_m for unstressed, undamaged plants was $0.14 \leq F_0/F_m \leq 0.20$ as is observed here. Bilger *et al*. (1987) and other researchers report that F_0/F_m is markedly increased for stressed or damaged plants. The principal cause of this at a steady F_0/F_m can be attributed to safe dissipation; it may be harmful to PS2 due to over-reduction of reaction centres (Demmig-Adams and Adams 1992). According to studies on salt-sensitive species like *Brassica* (*B. juncea* Coss) and sweet almond (*Prunus dulcis* Miller), NaCl induced an increase in F_0 and a decrease in F_m resulting in a significant increase of F_0/F_m (Misra *et al.* 2001, Ranjbarfordoei *et al.* 2006). Therefore, F_0 showed a better correlation with the disease index exhibited by plants and also with salinity dose than the parameter F_v/F_m (Sixto *et al.* 2006). Thus, the higher F_0 values, related to the degradation of D1 protein in PS2 (Aro *et al.* 1994) or associated with dissociation of the light-harvesting Chl *a*/*b* complexes from the reaction centre complex of PS2 (Yamane *et al.* 2000) could be a good predictor for salt sensitivity (Sixto *et al.* 2006). Our results showing non-significant increase in F_0 in *C. maritima* (independently of their origin) subjected to salt plead in favour of this assumption.

We did not find significant changes in Φ_{PS2} and ETR in Jerba plants. Both parameters were affected only at S_{400} in Tabarka. Thus the stomatal limitations imposed on photosynthesis may be accompanied by a decrease in the rate of consumption of ATP and NADPH for CO_2 assimilation, which can result in decreases in the rate of linear electron transport and, consequently, in Φ_{PS2} (Baker and Rosenqvist 2004). However, in C_3 plants an increase in photorespiration under the stress may maintain rates of electron transport similar to those observed in non-stressed leaves despite decreasing P_N (Leegood and Edwards 1996, Flexas *et al*. 2002, Noctor *et al*. 2002). This would result in little or no change in Φ_{PS2}.

We observed that P_N decreased significantly with the increase in salt concentration (Fig. 4). The lower P_N of salt-adapted plants means that they received excess of photons. However, the fact that photoinhibition was not enhanced in irradiated salt-adapted plants as observed in this study suggests mechanisms by which excess energy is dissipated safely in salt-adapted plants. NPQ is often used as indicator of the excess radiant energy dissipation to heat in the PS2 antenna complexes (Bilger and Björkman 1990, Demmig-Adams *et al*. 1996, Gilmore 1997). As NPQ did increase significantly in response to higher salt stress (Table 2), this radiant energy dissipation process (NPQ) may be an energy dissipation mechanism that protects the photosynthetic apparatus against excess photon energy (Qiu *et al.* 2003). In addition, an increase in NPQ may be related to the contribution of different relaxation-time components (Keiller *et al.* 1994, de Mattos *et al.* 1999).

In conclusion, *C. maritima* is one of the most promising halophytes in Tunisia and other countries, mainly for industrial purposes. Indeed, oilseed properties are comparable to those of oleaginous crops, such as rape with erucic acid as prominent fatty acid (more than 25 %

of total fatty acids; Ghars *et al*. 2006). Some ecotypes, like Jerba, were particularly tolerant to salt. We found that the leaves of *C. maritima* accumulated high amounts of salt. However, as discussed above, PS2 of *C. maritima* showed high tolerance to salinity up to S_{400}, indicating that either Na^+ or Cl^- had no toxic effects on PS2 photochemistry. This can be explained by the fact that Na^+ and Cl^- were compartmentalized in the vacuoles (Debez *et al*. 2006).

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