Biosaline Agriculture and Salinity Tolerance in Plants
Edited by M. Öztürk, Y. Waisel, M.A. Khan and G. Görk
© 2006 Birkhäuser Verlag/Switzerland

Salt effect on growth, photosynthesis, seed yield and oil composition of the potential crop halophyte *Cakile maritima*

Ahmed Debez[1], Wael Taamalli[2], Dhouha Saadaoui[1], Zeineb Ouerghi[1], Mokhtar Zarrouk[2], Bernhard Huchzermeyer[3] and Chedly Abdelly[1]

[1] *Laboratoire d'Adaptation des Plantes aux Stress Abiotiques, INRST, BP 95, Hammam-Lif, 2050, Tunisia*
[2] *Laboratoire de Caractérisation et Qualité de l'Huile d'Olive, INRST, BP 95, Hammam-Lif, 2050, Tunisia*
[3] *Institut für Botanik, Universität Hannover, Herrenhäuserstr. 2, 30419 Hannover, Germany*

Introduction

Salinity is an extending environmental issue which compromises the long-term sustainability of agriculture, especially in the coastal semi-arid areas [1]. This is the case in Tunisia, where the semi-arid Mediterranean climate prevails (mean annual precipitation of 200–700 mm). Subsequently, around 10 % of the whole territory would be salt-affected [2]. Halophytes have evolved a wide range of attributes (morphological, physiological and biochemical) allowing them to tolerate the presence of salt in the medium [3]. Besides, several studies suggest that these plants are potentially useful for ecological and economical purposes [4].

Intracellular salt flux control is one of the major salt tolerance determinants, involving salt exclusion and/or compartmentation. P and V H^+-ATPases (respectively localized at the plasma membrane and tonoplast) provide energy for Na^+/H^+ antiporters, thus allowing sodium active transport away from the cytoplasm [5]. The bi-directional transport of sodium insures ion homeostasis, cell turgor, as well as the metabolic functioning [6]. On the other hand, impairment of the photosynthetic activity greatly accounts for growth restriction of non-halophytes under salinity [7]. Depressive effects of salinity are thought to arise from stomatal and/or non stomatal limitations (i.e., stomatal closure and/or damage to Calvin cycle enzymes) [8].

Cakile maritima (*Brassicaceae*) is an annual fleshy halophyte which colonizes the sandy beaches of the Tunisian littoral. This study aims to characterize the plant response to long-term salt treatments (0–500 mM NaCl), using physiological (growth, water status, mineral nutrition) and biochemical (H^+-ATPase activity and photosynthetic capacity) criteria. Changes in seed yield and seed oil characteristics under salinity are also assessed.

Material and methods

Mature seeds of *C. maritima* were harvested on sandy beaches of Raoued (20 km to the north of Tunis). Seedlings were grown in pots filled with inert sand in a glasshouse (16 h/8 h light/dark regime; 60 % relative humidity; 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic active radiation – PAR; $22 \pm 1^{\circ}$ C temperature). Irrigation was performed with a Long Ashton nutrient solution [9]. Four week-old plants were progressively submitted to increasing salinities (0–500 mM NaCl) for 6 weeks. The same experience was repeated until the complete maturation of seeds. At the final harvest, physiological parameters were determined (i.e., dry weight, leaf number and area, leaf succulence ratio, leaf ion status). Yield components assessed were seed yield, seed mass, seed viability, and seed oil content.

Expanded leaves situated on the fifth node from the shoot top were used for photosynthetic and H^+-ATPase activity measurements. Leaf gas exchanges were measured with a portable photosynthesis system (LCi, ADC Bioscientific Ltd., UK) at 2,500 $\mu mol.m^{-2}.s^{-1}$ PAR (saturating light). Ribulose-biphosphate carboxylase/oxygenase (Rubisco; EC 4.1.1.39) activity was spectrophotometrically assayed (λ = 340 nm) [10]. Vacuolar and plasma membrane (respectively, V and P) H^+-ATPase activities were assayed on isolated chloroplasts, using [$\gamma - ^{32}$P] ATP (1 MBq) (Hartmann Analytik, Braunschweig, Germany) [11]. Seed total lipids were extracted [12] and triacylglycerols (TAG) were separated by thin layer chromatography (TLC), using silica gel plates (Merck G 60) [13]. Fatty acid methyl esters were quantified by adding heptadecanoic acid (17:0) as an internal standard. Results are the means of three samples. A one way ANOVA was achieved to compare the mean values, using the SPSS statistical program ($P < 0.05$). In case of significant differences, Duncan *post hoc* tests were performed.

Results and discussion

Moderate salinities (50–100 mM NaCl) were optimal for the plant growth, since improving whole plant dry weight (+24 % at 100 mM NaCl) (Fig. 1A). No significant growth decrease occurred in the 200–300 mM NaCl range (ca. 90 % of control values), and the plant was able to survive, even at a salinity close to that of seawater (500 mM NaCl). These data corroborate previous investigations on other halophytes, showing sub-optimal growth in mediums lacking salt [14, 15]]. Leaves largely accounted for the plant response pattern, since their dry weight and number were significantly stimulated at optimal salinities (50–100 mM NaCl) (Figs 1A and 1B). Leaf water status, evaluated by leaf succulence ratio, was significantly enhanced by salt treatments (Fig. 2A), and remained higher than the control values, even at 500 mM NaCl. The improvement of leaf hydration under salinity was concomitant with the accumulation of high amounts of Na^+, and at a lesser content of Cl^- (Fig. 2B) (1.8 and 3.8 $mmol.g^{-1}$ DW, respectively). Since salt treatment did not impair leaf hydration, most of Na^+ ions transported in leaves might have been removed from the leaf apoplast and efficiently compartmentalized by cells for water retention. Salinity restricted the plant nutrient uptake, leading to a significant decrease in leaf K^+

contents (Fig. 2B). The same tendency was observed for Ca^{2+} and Mg^{2+} (data not shown). The salt-induced reduction of growth could be a consequence of nutritional imbalance. Moreover, despite Na^{+} is a cheap osmoticum for halophytes, an excess of this ion over K^{+} can inhibit several metabolic processes.

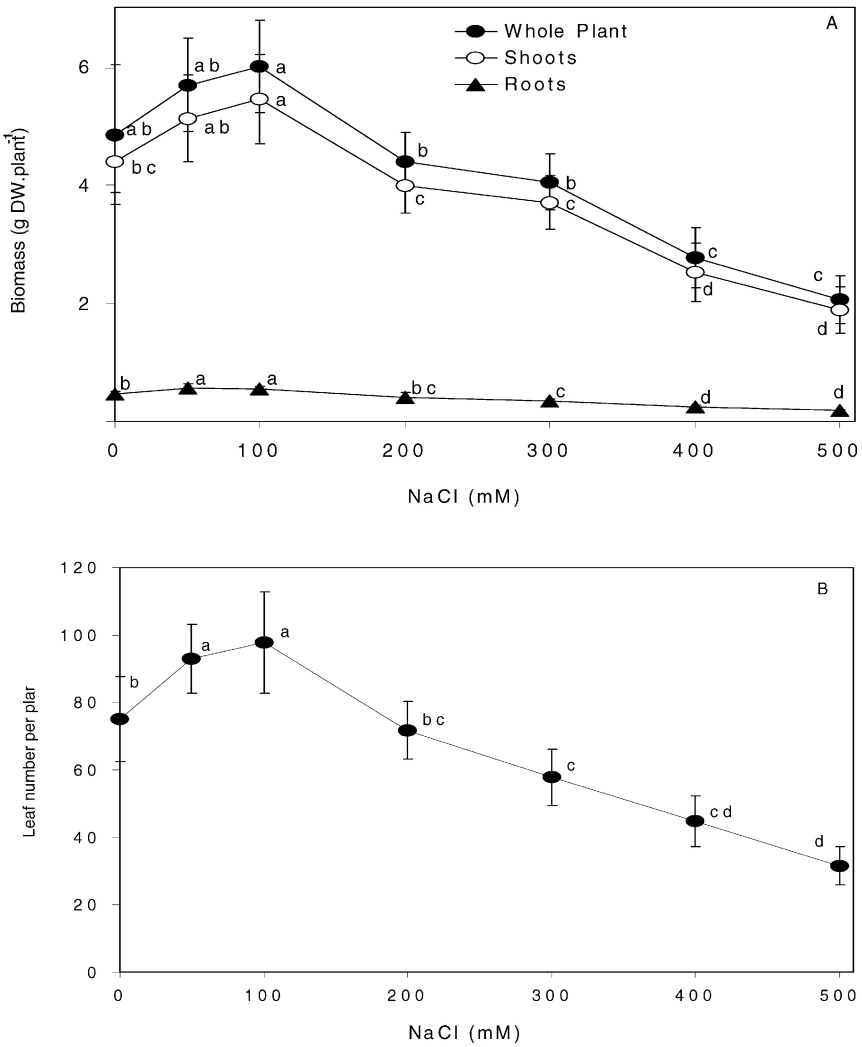

Figure 1. Effect of NaCl on growth of *C. maritima*. (**A**) Biomass production of the whole plant and the different organs. (**B**) Leaf number per plant. Means of 18 replicates ± SE. (Values within each salt treatment marked with at least one same letter are not significantly different at $P < 0.05$.)

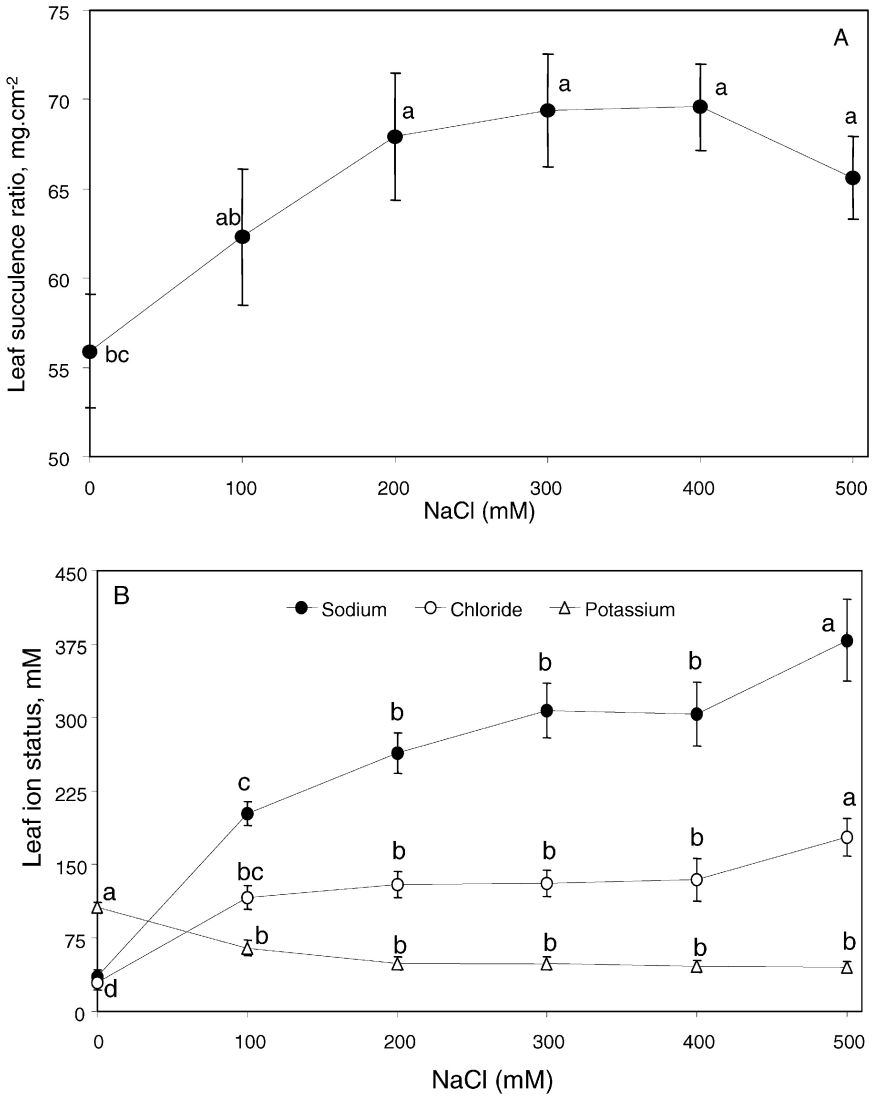

Figure 2. Effect of NaCl on leaf water status and mineral nutrition of *C. maritima*. (**A**) Leaf succulence ratio. (**B**) Leaf ion contents. Means of 18 replicates ± SE. (Values within each salt treatment marked with at least one same letter are not significantly different at $P < 0.05$.)

Combining the results relative to the leaf water status and salt accumulation of salt-treated *C. maritima* provide indirect evidence for the existence of salt compartmentation mechanisms within leaf cells (i.e., inclusive strategy). This assumption was confirmed by (i) the strong stimulation of V H^+-ATPase activity up to 300 mM NaCl (+80 %/control) (Fig. 3A) and (*ii*) the absence of anatomical structures

responsible for salt exclusion at the leaf surface. NaCl concentrations in the 300–500 mM range significantly promoted P H^+-ATPase activity, suggesting that the exclusive pattern may take place at high salinities (Fig. 3B). Keeping sodium and chloride away from cytosol (using inclusive strategy) is of vital importance for dicotyledonous halophytes lacking morphological structures of salt excretion at their leaf surface [16]. Owing to their catalyzer role, proton pumps enable both vacuolar and plasma membrane antiporter functioning, and play therefore, a major role in salt tolerance [17]. In addition, the overexpression of Na^+/H^+ antiporters plants has been reported to improve the performance of several species in saline conditions [18].

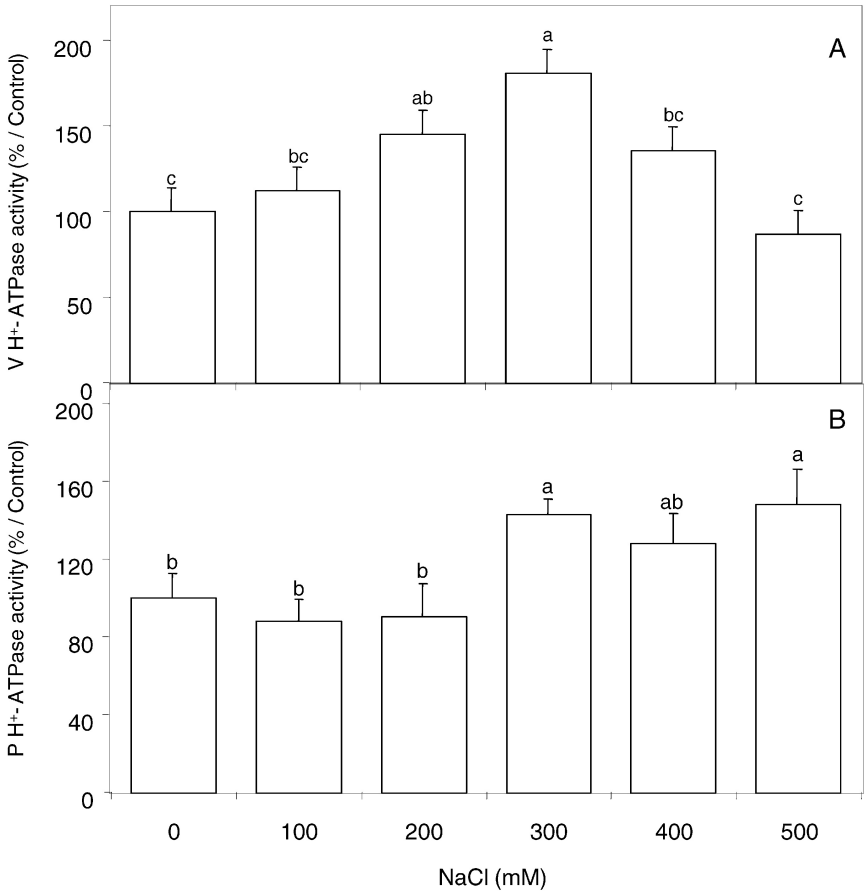

Figure 3. Effect of NaCl on H^+-ATPase activity (%/Control) of *C. maritima*. (**A**) Changes in vacuolar V H^+-ATPase activity. (**B**) Changes in plasma membrane P H^+-ATPAse activity. Means of three replicates $\pm$ SE. (Values within each salt treatment marked with at least one same letter are not significantly different at $P < 0.05$.)

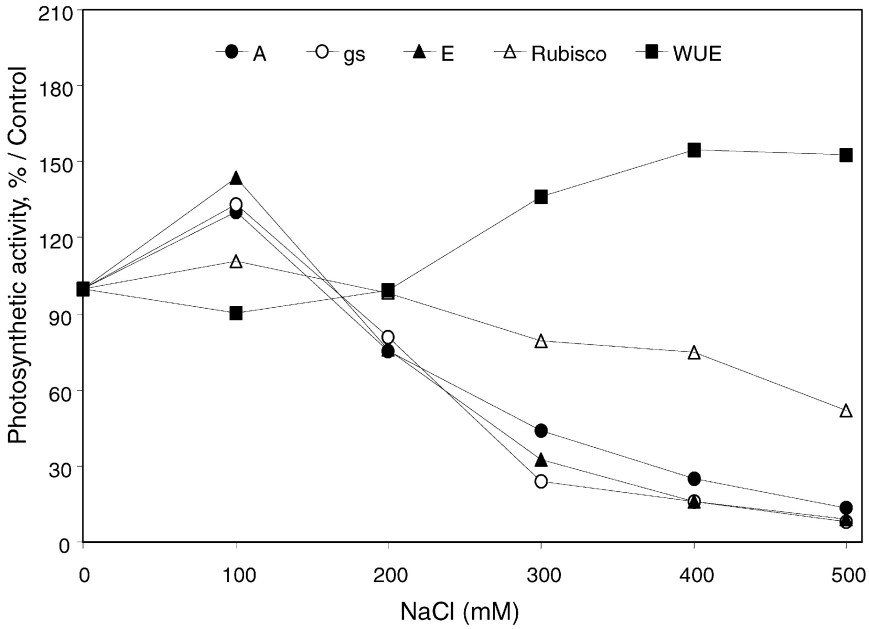

Figure 4. Effect of NaCl on photosynthetic activity of *C. maritima*. Results of gas exchanges are the means of 10 replicates. Results of Rubisco activity are the means of three replicates.

Both stomatal and non stomatal components of photosynthesis were improved at optimal salinity for growth. CO_2 assimilation rate (A), stomatal conductance (g_s), and transpiration rate (E) were 30–40 % higher at 100 mM NaCl, while specific activity of Rubisco was augmented by ca. 10 % (Fig. 4). Supra-optimal salinities impaired photosynthetic activity, but this depressive effect was more pronounced on stomatal conductance than on enzyme activity (respectively 15 % and 75 % of the control values at 400 mM NaCl). Former studies showed that stomatal limitation accounted for the reduction of photosynthesis in salt-treated plants [8]. In *C. maritima*, stomata closure was associated with reduced transpiration rate (E), leading to higher water-use efficiency (+ ca. 50 % at 500 mM NaCl). No salt-induced shift in the photosynthetic pathway (C_3 to C_4) was observed, since phosphoenolpyruvate carboxylase (PEPC, EC 4.1.1.39) activity remained lower than Rubisco one, irrespective of salt treatment (data not shown).

Optimal salinities for growth and photosynthesis promoted significantly seed production (+50 % in the 50–100 mM NaCl range) (Fig. 5A). This parameter was more affected than plant growth at high salinities (respectively 21 % and 84 % of the control values at 300 mM NaCl), likely resulting from a reduction of flower production and/or a decrease of their fertility [19]. The mean mass of individual seed decreased significantly in the presence of salt in *C. maritima* (Fig. 5A), indicating that assimilate allocation to seeds was more restricted by salt than seed initiation. Seeds harvested from plants exposed to mild salinities (50–200 mM NaCl) displayed

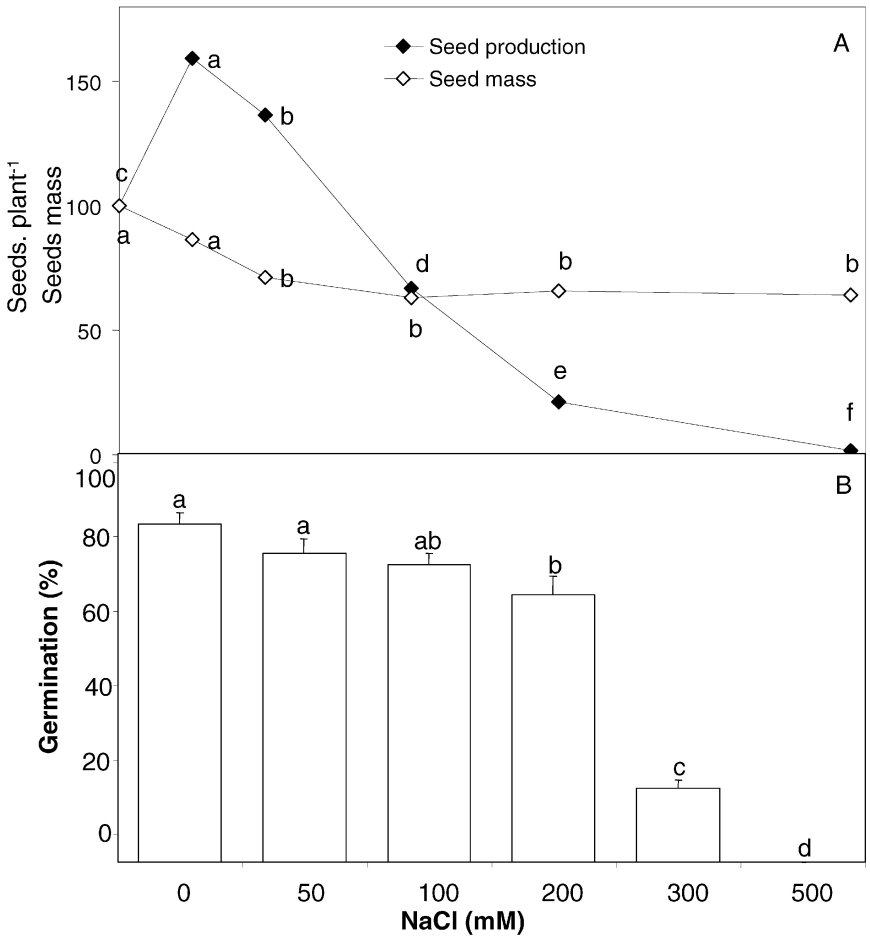

Figure 5. Effect of NaCl on the reproductive capacity of *C. maritima*. (**A**) Seed production per plant (means of 12 replicates) and individual seed mass (mg) (means of 300 replicates), expressed as % of the control. (**B**) Germination capacity (%) in distilled water of seeds harvested from plants exposed to increasing salinities. Means of three replicates ± SE. (Values within each salt treatment marked with at least one same letter are not significantly different at $P < 0.05$.)

high germination rates (up to 80 %), contrasting with those produced under high salt levels (Fig. 5B). Increasing salinities led to both quantitative and qualitative changes in the seed oil characteristics. Seed oil content (on a dry weight basis) was positively correlated with the medium salinity (respectively 30 % and 28 % at 100 mM and 500 mM NaCl). Seed oil content seemed also to be unaffected by salinity in the oleaginous halophyte *Lesquerella fendleri* [20], while decreasing in sunflower [21]. Erucic acid (22:1) level increased markedly, reaching 26 % at 500 mM NaCl (two-fold higher than control values). This trend was concomitant with a significant decrease in oleic

acid (18:1) level (ca. 45 % of the control value at 500 mM NaCl). In our conditions, higher erucic acid in salt treated *C. maritima* was associated with increased 22:1/18:1 ratio (0.39 and 1.36, respectively for the control and 500 mM NaCl plants), likely mediating elongases, which are known to catalyze the formation of long fatty acids (such as erucic acid), using oleic acid as initial substrate [22].

In summary, the present study shows that moderate salinities are required by *C. maritima* to express maximal growth and seed production potentialities, in relation with the concomitant involvement of several processes at different levels. Further field experiments are necessary to confirm the economic potential of this promising halophyte.

Acknowledgements

This work was supported by a sandwich scholarship granted by the German Academic Exchange Service (Deutscher Akademischer Austausch Dienst, DAAD). We wish to thank Professor Ahlert Schmidt (Institut für Botanik, University of Hannover, Germany) for his kind collaboration and for providing technical facilities.

References

1 Horie T, Schroeder JI (2004) Sodium transporters in Plants. Diverse genes and physiological functions. *Plant Physiol* 136: 2457–2462
2 Hachicha M, Job JO, Mtimet A (1994) Les sols salés et la salinisation en Tunisie. *Sols de Tunisie* 5: 271–341
3 Glenn E, Brown JJ, Blumwald E (1999) Salt-tolerant mechanism and crop potential of halophytes. *Crit Rev Plant Sci* 18: 227–255
4 Glenn EP, O'Leary JW, Watson MC, Thompson TL, Kuehl RO (1991) *Salicornia bigelovii* Torr.: an oilseed halophyte for seawater irrigation. *Science* 251: 1065–1067
5 Zhu JK (2001) Plant salt tolerance. *Trends Plant Sci* 6: 66–71
6 Aharon GS, Apse MP, Duan S, Hua X, Blumwald E (2003) Characterization of a family of vacuolar Na^+/H^+ antiporters in *Arabidopsis thaliana*. *Plant Soil* 253: 245–256
7 Munns R (2002) Comparative physiology of salt and water stress. *Plant Cell Environ* 25: 239–250
8 Meloni DA, Oliva MA, Martinez CA, Cambraia J (2003) Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. *Environ Exp Bot* 49: 69–76
9 Abdelly C, Lachaâl M, Grignon C, Soltani A, Hajji M (1995) Association épisodique d'halophytes strictes et de glycophytes dans un écosystème hydromorphe salé en zone semiaride. *Agronomie* 15: 557–568
10 Sato FK, Nishida K, Yamada Y (1980) Activities of carboxylation enzymes and products of $^{14}CO_2$ fixation in photoautotrophically cultured cells. *Plant Sci Lett* 20: 91–97
11 Koyro H-W, Stelzer R, Huchzermeyer B (1993) ATPase activities and membrane fine structure of rhizodermal cells from *Sorghum* and *Spartina* roots grown under mild salt stress. *Bot Acta* 106: 110–119
12 Allen C, Good P (1971) Acyl lipids in photosynthetic systems. *Methods in Enz* 23: 523–547
13 Mangold HK (1964) Thin layer chromatography of lipids. *JAOCS* 47: 726–773
14 Bajji M, Kinet JM, Lutts S (1998) Salt stress effects on roots and leaves of *Atriplex halimus* L. and their corresponding callus cultures. *Plant Sci* 137: 131–142

15 Short DC, Colmer TD (1999) Salt tolerance in the halophyte *Haloscaria pergranulata* subsp. *pergranulata*. *Ann Bot* 83: 207–213
16 Véry A-A, Robinson MF, Mansfield TA, Sanders D (1998) Guard cell cation channels are involved in Na^+-induced stomatal closure in a halophyte. *Plant J* 14: 509–521
17 Zhu J-K (2003) Regulation of ion homeostasis under salt stress. *Curr Opin Plant Biol* 6: 441–445
18 Venema K, Belver A, Marin-Manzano MC, Rodriguez-Rosales MP, Donaire JP (2003) A novel intracellular K^+/H^+ antiporter related to Na^+/H^+ antiporters is important for K^+ ion homeostasis in plants. *J Biol Chem* 278: 22453–22459
19 Khatun S, Flowers TJ (1995) The estimation of pollen viability in rice. *J Exp Bot* 46: 151–154
20 Dierig DA, Grieve CM, Shannon MC (2003) Selection for salt tolerance in *Lesquerella fendleri* (Gray) S. Wats. *Indust Crops Prod* 17: 15–22
21 Flagella Z, Giuliani MM, Rotunno T, Di Caterina R, De Caro A (2004) Effect of saline water on oil yield and quality of a high oleic sunflower (*Helianthus annuus* L.) hybrid. *Eur J Agron* 21: 267–272
22 Katavic V, Mietkiewska E, Barton DL, Giblin EM, Reed DW, Taylor DC (2002) Restoring enzyme activity in nonfunctional low erucic acid *Brassica napus* fatty acid elongase 1 by a single amino acid substitution. *Eur J Biochem* 269: 5625–5631