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Impact of Repetitive Salt Shocks on Seedlings of the Halophyte *Cakile maritima*

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Salinization is one of the main environmental constraints that threat global crop biomass production. Halophytic species are currently widely studied because of their value for the development of saline agriculture. In this study we investigated the impact of repetitive salt shocks to mimic inundation with seawater and recorded changes in ionic and water status, growth parameters and some markers of oxidative stress of *sea rocket*, *Cakile maritima*, succulent halophyte which displays potential for economical nutrient food, for therapeutic utilization and for it seeds contain up to 40% of oil. *Cakile maritima* displayed different growth behavior in response to salt shock. Repetitive salt shocks with NaCl concentrations superior to 200 mM induced a reduction of growth of aerial parts. For the lower concentrations of NaCl (50–100 mM), we observed slight but not significant growth stimulation. Under salt shock, *C. maritima* maintained hydration in the aerial parts despite their high contents in sodium and chlorine. Changes of malondialdehyde (MDA) and ascorbic acid under salt shock revealed beneficial and damaging functions of these molecules at low and high salt shock, respectively.

Keywords : antioxidant, *Cakile maritima*, halophytes, salt shock

INTRODUCTION

Salinization is one of the main environmental constraints that threat global crop biomass production and thus food security. Hence halophytic species are currently widely studied in world because of their value for the development of saline agriculture. However, when applying salt two distinct protocols could be used salt stress and salt shock leading to different responses (Shavrukov, 2012). Gradual salt application (usually of 25 or maximum 50 mM increments of NaCl, until a final, predetermined salt concentration is reached) reflects the reactions expected for salt stress in saline field environments (Shavrukov et al., 2012). Various researchers refer to this method as progressive imposition (Almansouri et al., 1999), salt acclimation, gradual step acclimation (Rodriguez et al., 1997; Sanchez et al., 2008), or salt-adapting (Baisakh et al., 2006). Most of plants in natural ecosystems risk salt stress, but coastal halophytes growing on sand dunes are subject to inundation with seawater (Davy, 2006) and thus repetitive salt shocks. The main component of salt shock is osmotic shock inducing plasmolysis, especially in root cells (Munns, 2002). Desperate attempts by the cells to maintain equilibrium between external and internal water content results in the leakage of cell solution into open spaces between the cell wall and plasma membrane. These apoplastic solutes, containing high concentrations of Na⁺, can freely flow through the open spaces in root cells and be transported to the shoot with minimal control by the plant. There is consequently rapid activation of many genes, in response to osmotic

shock and damaged plasma membrane in root cells and to ionic stress in shoot cells (Shavrukov et al., 2012).

High cellular NaCl concentrations are also supposed to increase formation of reactive oxygen species (ROS) (Hernandez and Almansa, 2002), which is considered as a primary event under a salt stress conditions (Noctor and Foyer, 1998). ROS could damage photosynthetic components, inactivate proteins and enzymes, and permeabilize membranes by causing lipid peroxidation (Price and Hendry, 1991; Meloni et al., 2003). Plants with high levels of anti-oxidants, either constitutive or induced, have been reported to have greater resistance to this oxidative damage (Shalata and Tal, 1998; Bor et al., 2003). Such correlation between anti-oxidant capacity and salt tolerance has also been demonstrated in a large number of plants, including salt-tolerant glycophytes and true halophytes (Broetto et al., 2002; Bor et al., 2003; Agarwal and Pandey, 2004; Ben Amor et al., 2005).

Sea rocket or *Cakile maritima* (*Brassicaceae*) is an annual, succulent halophyte widely distributed in sandy coasts throughout the world (Clausing et al., 2000) and thus subjected to salt shocks. This plant displays potential for economical nutrient food (leaf comestible), for therapeutic utilization (Casal, 2004) and for it seeds contain up to 40% of oil (Ghars et al., 2005). Since at this time most of the studies on *C. maritima* were made using gradual salt application (Ben Amor et al., 2005; Debez et al., 2006; Ellouzi et al., 2014), we investigated the impact of repetitive salt shocks to mimic inundation with seawater and recorded changes in ionic and water status, growth parameters and some markers of oxidative stress in different organs

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(leaves, stems and roots) of *C. maritima* seedlings.

MATERIALS AND METHODS

Plant culture and treatments

Mature seeds of *Cakile maritima* (harvested at Raoued, a locality close to the Mediterranean seashore, 20 km north of Tunis) were germinated directly in pots filled with sand, seedlings were irrigated for 3 weeks with Long Ashton nutrient solution (Debez et al., 2004). After, plants were separated in six groups and irrigated repetitively three times per week with a nutrient solution supplemented with different concentrations of NaCl (0, 50, 100, 200, 300 and 400 mM). The experiments were performed in a glasshouse under controlled conditions, photoperiod of 16/8 h (light/darkness), relative humidity ranging 60%, photosynthetically active radiations of $400 \mu\text{mol m}^{-2} \text{s}^{-1}$. The final harvest occurred after 21 days of repetitive shocks.

Growth and water status

The fresh weight (FW) and dry weight (DW) of leaves, stems and roots were measured at each sampling. Shoot or root water content was calculated as $(\text{FW} - \text{DW})/\text{FW}$.

Gas exchange measurements and chlorophyll content

After 21 days of salt shock imposition, the net CO_2 assimilation rate (A), stomatal conductance (g_s), and leaf intercellular CO_2 concentration (C_i) were determined. Stomatal conductance (g_s), and transpiration rate (E) were determined by using a portable photosynthesis system (LCA4, LI-COR Inc., USA). Measurements were carried out between 10:00 and 12:00 a.m. on leaves (10 replicates per treatment). Data were automatically collected every minute after photosynthesis rate was stabilized.

The concentrations of photosynthetic pigments were estimated by method described by Torrecillas et al. (1984).

Determination of Na^+ , K^+ and Cl^- concentrations

Na^+ and K^+ were extracted from the desiccated shoot, stems and root tissues with 0.5% (v/v) nitric acid and were assayed by flame emission photometry (Ciba, Corning, UK). Chloride was determined in neutral medium by a standard solution of silver nitrate in the presence of potassium chromate (Kraemer and Stamm, 1924). The end of the reaction was indicated by the appearance of the red color characteristic of silver chromate.

The selectivity of ion accumulation for K^+ over Na^+ was estimated from ion contents using the following ratio: $S = (\text{K}^+ / (\text{K}^+ + \text{Na}^+))_{\text{leaves}} / [\text{K}^+ / (\text{K}^+ + \text{Na}^+)]_{\text{medium}}$.

Lipid peroxidation

The extent of lipid peroxidation was estimated by determining the concentration of malondialdehyde (MDA). The analysis method was to homogenize fresh material in the TCA acid 0.1% (w/v). The homogenate was centrifuged at $13\,000 \times g$ for 30 min. The supernatant was recovered and incubated in a mixture (TBA (0.5%)/TCA (20%)) (v/v) at 95°C for 30 min, then cooled in ice to stop the reaction. Centrifugation was then carried out at $10\,000 \times g$ for 10 min. The supernatant was used for reading the absorbance at 532 nm. The rate of TBARS was calculated

based on the molar extinction coefficient of $155 \text{ mM}^{-1} \text{ cm}^{-1}$ (Hagège et al., 1990).

Quantification of H_2O_2

The concentrations of H_2O_2 were quantified following the method reported previously (Ferguson et al., 1983), fresh plant material were homogenized in cold acetone, the mixture was incubated for 30 min at 4°C , then filtered, after the addition of activated carbon, the mixture was centrifuged for 20 min at $3000 \times g$ at 4°C , then TiCl_4 20% HCl (v/v) and 0.2 mL of NH_4OH were added successively to the supernatant. The post-reaction compound was centrifuged for 10 min at $3000 \times g$ at 4°C , the pellet recovered was dissolved in 2 mL of H_2SO_4 (1M). The absorbance reading was realized at 410 nm and H_2O_2 content was calculated using a standard curve with concentration ranging from 0.1 to 1 mM.

Statistics

All data were subjected to an analysis of variance (ANOVA). Mean separation was accomplished by the Newman and Keuls multiple range test and significance was evaluated at the probability level of $P \leq 0.05$.

RESULTS

Growth and tissue hydration

Repetitive salt shocks with moderate concentrations of NaCl (50–100 mM) did not significantly affect the growth of *C. maritima* when higher concentrations seemed to decrease the biomass of the leaves and stems (Fig. 1A, B). At 400 mM NaCl, the reduction of growth estimated by DW was estimated at 36% of the control (Fig. 1B). No significant change on roots growth in different parts of plant was observed under repetitive salt shocks (Fig. 1B). However plants remained alive even at the most extreme salt concentration (Fig. 1A). Leaf and stem hydration levels were not significantly affected by salinity (Fig. 2) although data showed the same trends as the one observed for growth, ie. no effect of repetitive salt shocks until 200 mM NaCl and then a decrease for higher concentrations (Fig. 2). At the highest salt level (400 mM NaCl), leaf and stems water content were respectively at 85% and 83% of the control value. The root water content was not impaired in the range of salinity tested (Fig. 2).

Ionic status

As expected for a salt inclusive halophyte (Debez et al., 2006), large amounts of Na^+ were transported to the leaves. For instance, Na^+ accumulation in these organs reached $4.8 \text{ mmol g}^{-1} \text{ DW}$ at the highest NaCl concentration tested (Fig. 3A). Accumulation of Na^+ in leaves of *C. maritima* is dose-dependent (Fig. 3A). A similar pattern was observed, though at a lesser extent in stems. Concerning Cl^- accumulation in leaves and stems, a similar pattern was also observed but Cl^- was more accumulated in stems than in leaves (Fig. 3B). In roots no significant changes were observed in Na^+ and Cl^- concentrations (Fig. 3A).

Leave and stem accumulations after repetitive salt shocks were concomitant with a maintaining of K^+ amounts similar to control (Fig. 3C). To assess the ability

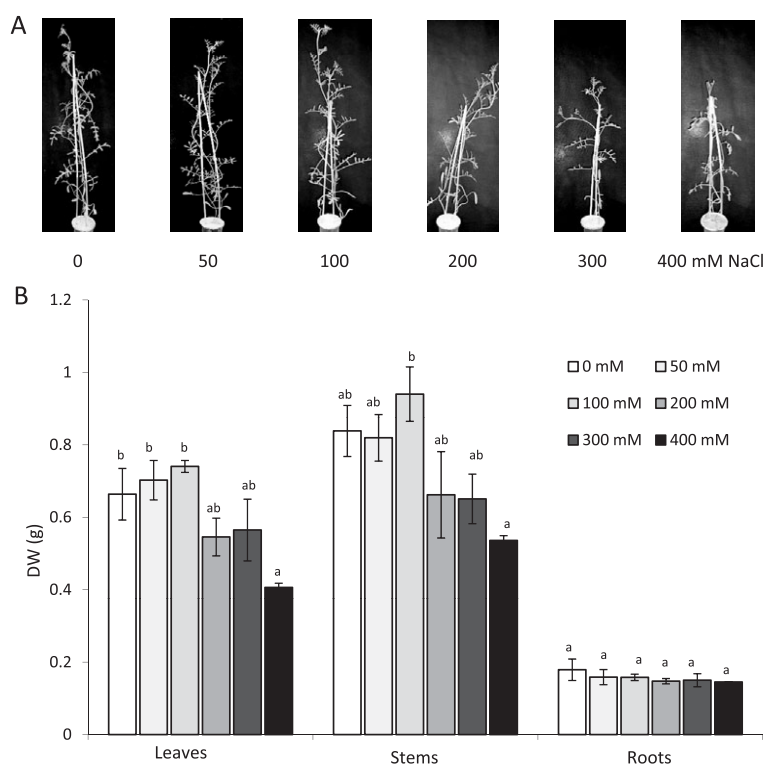


Fig. 1 Variation of biomass production of seedlings from three weeks after repetitive salts shocks from 50 to 400 mM NaCl. (A) Pictures of seedlings. (B) Biomass of leaves stems and roots. The data reflect the means \pm SE of at least 3 independent replicates. Columns with the same letters are not significantly different at $P \leq 0.05$.

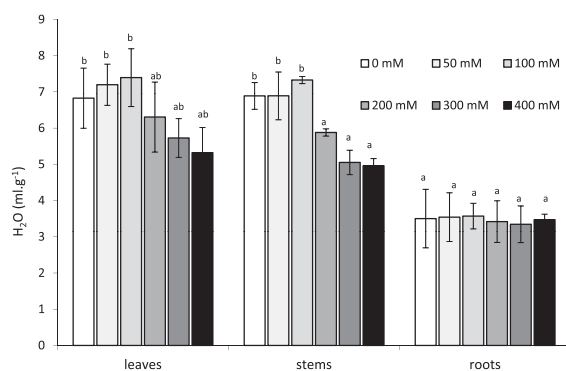


Fig. 2 Variations in water content of *Cakile maritima* seedlings from three weeks after repetitive salt shocks (50 to 400 mM NaCl). Data reflect the means \pm SE of at least 3 independent replicates. Columns with the same letters are not significantly different at $P \leq 0.05$.

of *C. maritima* to selectively uptake of K^+ in the presence of Na^+ excess a selectivity ratio S was calculated from leaf ion contents and ion concentrations in the medium. Results indicated a marked selectivity for K^+ over Na^+ with S values significantly increasing with salt treatments (Fig. 4).

Photosynthesis parameters

Repetitive salt shocks at 100 mM significantly increased the net CO_2 assimilation rate (A) by ca. 48% as compared to the control whereas for higher NaCl concentrations a trend to decrease was observed although not significant (Table 1). On the contrary, the transpiration rates (g_s) decreased in response to salt shocks (Table 1). Accordingly, the stomatal conductance (E) displayed a

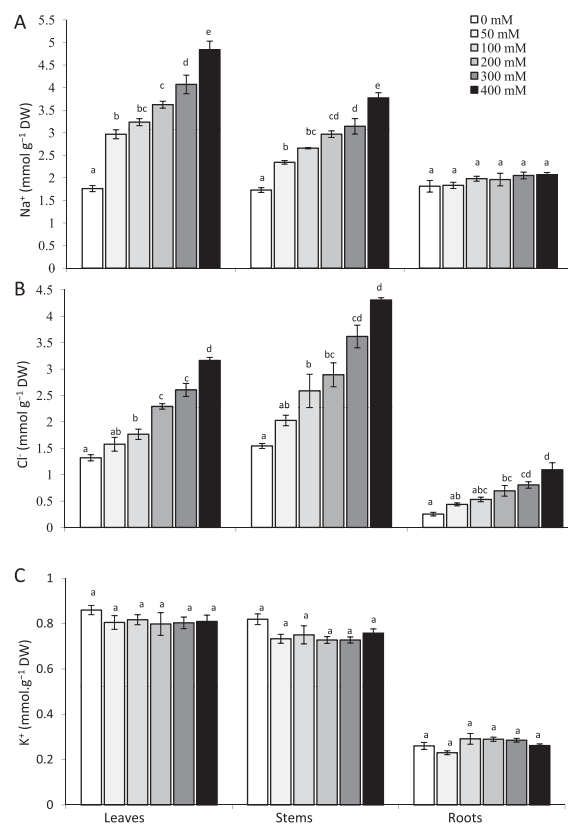


Fig. 3 Effect of repetitive salt shock treatments (three weeks) on Na^+ (A), Cl^- (B) and K^+ (C) concentrations in the leaves, stems and roots of *Cakile maritima*. The data reflect the means \pm SE of at least 3 independent replicates. Columns with the same letters are not significantly different at $P \leq 0.05$.

Table 1 Changes in gas exchange parameters (net CO₂ assimilation rate (*A*), stomatal conductance (*g_s*), transpiration rate, (*E*), water use efficiency (WUE),) of *C. maritima* plants, exposed for 3 weeks to repetitive salt shock. The data reflect the means \pm SE of at least 3 independent replicates. In each columns values with the same letters are not significantly different at $P \leq 0.05$.

NaCl (mM)	<i>A</i> ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	<i>g_s</i> ($\text{mmol m}^{-2} \text{s}^{-1}$)	<i>E</i> ($\text{mmol m}^{-2} \text{s}^{-1}$)	WUE
0	$2.840 \pm 0.006^{\text{ab}}$	$87 \pm 19^{\text{c}}$	$1.933 \pm 0.142^{\text{b}}$	$1.48 \pm 0.120^{\text{a}}$
50	$3.365 \pm 0.108^{\text{b}}$	$67 \pm 5^{\text{bc}}$	$1.893 \pm 0.19^{\text{b}}$	$1.78 \pm 0.007^{\text{b}}$
100	$4.337 \pm 0.137^{\text{c}}$	$58 \pm 3^{\text{ab}}$	$1.680 \pm 0.004^{\text{b}}$	$2.58 \pm 0.118^{\text{b}}$
200	$3.527 \pm 0.214^{\text{b}}$	$56 \pm 14^{\text{ab}}$	$0.993 \pm 0.011^{\text{a}}$	$3.57 \pm 0.214^{\text{bc}}$
300	$2.470 \pm 0.129^{\text{a}}$	$51 \pm 9^{\text{a}}$	$1.013 \pm 0.003^{\text{a}}$	$2.44 \pm 0.198^{\text{b}}$
400	$2.557 \pm 0.007^{\text{a}}$	$50 \pm 3^{\text{a}}$	$0.897 \pm 0.204^{\text{a}}$	$2.97 \pm 0.707^{\text{b}}$

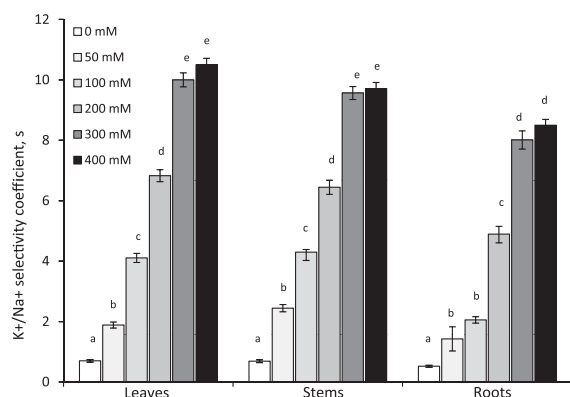


Fig. 4 Selectivity of ion accumulation in leaves, stems and roots. The selectivity for K⁺ vs Na⁺ was estimated from coefficient $S = [K^+/(K^+ + Na^+)]_{\text{leaves}}/[K^+/(K^+ + Na^+)]_{\text{medium}}$. Using ion contents of 3 weeks at the indicated NaCl concentrations. Data reflect the means \pm SE of at least 3 independent replicates. Columns with the same letters are not significantly different at $P \leq 0.05$.

similar pattern. A significant decrease was observed in stomatal conductance and transpiration at 400 mM NaCl (ca. 42%, 54% of control, respectively). The WUE (ratio of *A* to *E*) increased with increase in salinity, the maximal value being reached with 200 mM NaCl. On the other hand, a significant reduction of leaf relative chlorophyll content was observed for concentrations superior to 200 mM NaCl (Fig. 5).

Lipid peroxidation

The extent of the salt-induced oxidative damage was assessed by measuring the MDA formation. At moderate salinity (100 mM NaCl) induced a significant increase of MDA levels in leaves and roots (ca. 39%, 104% of control value, respectively, (Fig. 6). At 400 mM NaCl the MDA concentration slightly decreased remaining significantly different from the control in leaves and roots (Fig. 6).

H₂O₂ accumulation

We thus tried to evaluate the cellular oxidative stress using H₂O₂ staining in leaves (Fig. 7) or biochemical quantification in the different part of the seedlings (Fig. 7) but failed to detect any increase in H₂O₂ whatever the NaCl concentrations tested.

Ascorbic acid content

In *C. maritima* roots, ascorbic acid levels increase under moderate salinity (ca. 75% of the control) then decrease at 400 mM NaCl and remained close to control

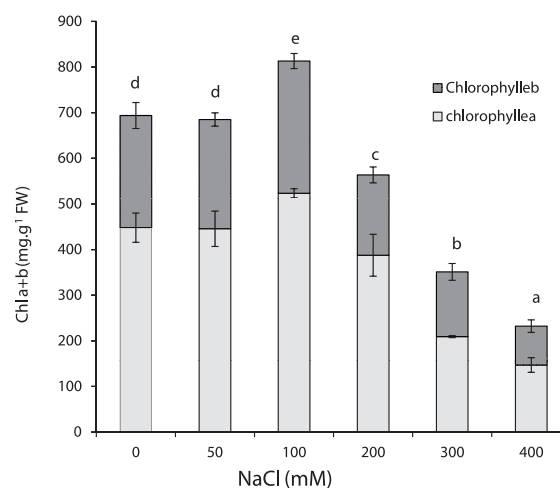


Fig. 5 Evolution of chlorophyll levels (Chla + b) in leaves of *Cakile maritima* after three weeks of repetitive salt shocks. Data reflect the means \pm SE of at least 3 independent replicates. Columns with the same letters are not significantly different at $P \leq 0.05$.

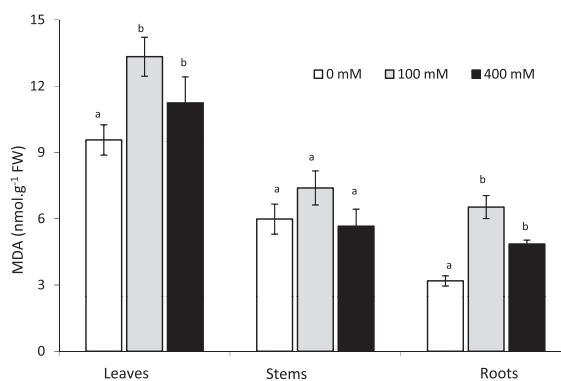


Fig. 6 Effect of repetitive salt shocks (100 and 400 mM NaCl) on MDA concentrations in the leaves, stems and roots of *Cakile maritima* seedlings from three weeks after repetitive salt shocks (100 or 400 mM NaCl). The data reflect the means \pm SE of at least 3 independent replicates. Columns with the same letters are not significantly different at $P \leq 0.05$.

levels. Although not significant a similar pattern was observed at a lesser extent in stems and leaves (Fig. 8). It is further noteworthy that in leaves the ascorbic acid level was strongly decreased at 400 mM NaCl (Fig. 8).

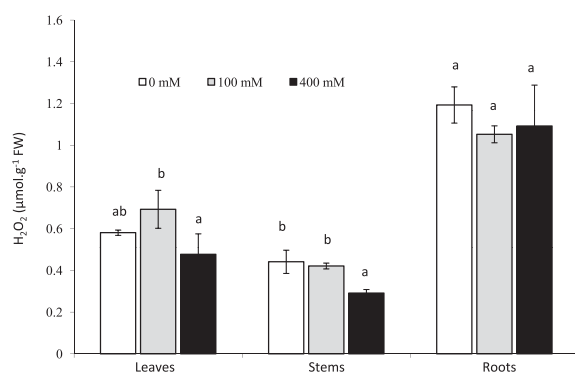


Fig. 7 Quantification of H₂O₂ from *Cakile maritima* seedlings of 3 weeks exposed to repetitive salt shocks (100 and 400 mM NaCl). The data reflect the means \pm SE of at least 3 independent replicates. Columns with the same letters are not significantly different at $P \leq 0.05$.

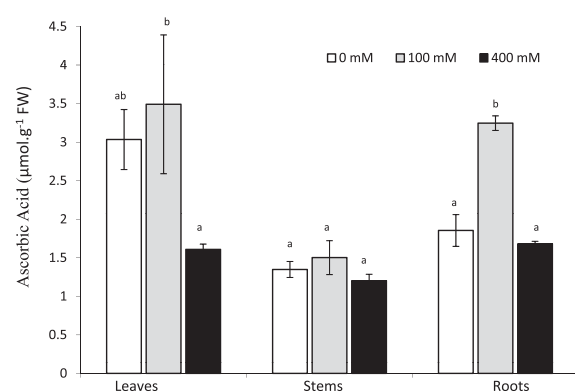


Fig. 8 Changes of ascorbic acid contents in the leaves, stems and roots of *Cakile maritima* seedlings from 3 weeks after repetitive salt shocks (100 or 400 mM NaCl). The data reflect the means \pm SE of at least 3 independent replicates. Columns with the same letters are not significantly different at $P \leq 0.05$.

DISCUSSION

Cakile maritima displayed different growth behavior in response to salt shock. Repetitive salt shocks with NaCl concentrations superior to 200 mM induced a reduction of growth of the aerial parts of *C. maritima*. It is noteworthy that the plants remained alive even at the most extreme salt concentrations. The same results were found under gradual salt application (Debez et al., 2004; 2006; Ben Amor et al., 2007; Megdiche et al., 2008). For the lower concentrations of NaCl (50–100 mM), these authors observed a growth stimulation. In our experiment this stimulation was slight but not significant. The growth of root system did not seem to be impacted by the salt shocks, suggesting that the salt was exported to the aerial parts.

In accordance with what was observed for growth, leaf hydration was not significantly affected upon repetitive salt shocks until 200 mM NaCl, but a slight decrease (significant only for the stems) was recorded with 400 mM NaCl. The root water content was not impaired in the range of salinity tested. Thus, this plant was able to maintain tissue

hydration upon both repetitive salt shocks (this study) and gradual salt application (Debez et al., 2004). This indicates that in case of halophyte studies, it is not always true that salt shock is accompanied with osmotic shock in plants.

Despite large amounts of Na⁺ and Cl⁻ added during a repetitive salt shock, distribution patterns and changes of Na⁺, Cl⁻ and K⁺ concentrations did not follow the same trend in roots, stems and leaves of *C. maritima*. Na⁺ and Cl⁻ were transported by roots and accumulated at high concentration in stem and leaf tissues of salt-treated plants. Our research showed that the sodium and chlorine content in leaves and stems increased with increasing salinity, especially at 400 mM NaCl (respectively 174% and 118% of control). Neither leaf dehydration nor severe leaf injury symptoms were observed, indicating that the plants did not endure toxic salt effects and suggest that efficient detoxification mechanisms are developed by *C. maritima* to tolerate the presence of Na⁺ inside the cells. *C. maritima* was able to prevent the accumulation of Na⁺ in the root cytoplasm and likely to transport it to leaf cells. Debez et al. (2006) had already showed that this halophyte is a typical Na⁺ includer, which efficiently compartmentalizes this ion within large vacuoles (Ben Hamed et al., 2014) for enhancing the water-retention capacity in salt-stressed plants (Munns and Passioura, 1984; Flowers and Yeo, 1986), whereas the potassium did not exhibit significant changes after 21 d of repetitive salt shock in the leaves, stems and roots of *Cakile maritima* (Fig 2). Potassium is an essential ion for various physiological processes; it is involved in protein synthesis and enzyme activation and it decreases transpirational water loss (Yu et al., 2011; Meychik et al., 2013). This plant appeared also to maintain tissue hydration by Na⁺ compartmentalization under gradual salt application (Debez et al., 2004). These results are in good agreement with our results, *C. maritima* did not show any water loss in leaves, stems and roots.

It is important to mention that salt stressed plant is due to two component: uptake and use of K⁺ under Na⁺ salinization, as well as the maintenance of high K⁺/Na⁺ ratio is essential for salt tolerance (Cromer et al., 1985). To assess the ability of *C. maritima* for selective uptake of K⁺ in the presence of Na⁺ excess, a selectivity ratio S was calculated from shoot and root ion contents and ion concentrations in the medium. The ratio S were higher in *C. maritima* leaves, stems and roots than in the culture medium, even at the highest external NaCl concentrations (300–400 mM). Increase in Na⁺/K⁺ ratio was due to Na⁺ interfering with K⁺ uptake which is essential for growth and for regulation of stomatal closure by a rapid release of K⁺ from the guard cells into the leaf apoplast (Marschner, 2012). In our result no significant change in potassium content in leaves and stems of *C. maritima* under repetitive salt shock.

The main component of salt shock is osmotic shock resulting from low substrate water potential (Munns, 2005). Ellouzi et al. (2014) show that after 4 h of salt shock, a transient dehydration observed in leaves and roots of *C. maritima*, without observation of irreversible damage, as well as the relative water content (RWC) increase after 72

h of salt stress. This transient decrease in water status, observed few hours after salt imposition, is an alert phase induced by the osmotic shock after salt stress perception (Aroca et al., 2012).

Salinity affects also photosynthetic parameters and induces photosynthesis inhibition coinciding with a strong decrease in the transpiration rate (E) and stomatal conductance (g_s) (Table 1), contributing to a positive water balance. Similar characteristics of water conservation have also been reported for *C. maritima* with progressive salt stress (Debez et al., 2006; Megdiche et al., 2008) and for many halophytic species under saline conditions (Carrol et al., 2001; Liu and Stützel, 2002; Elisa et al., 2012). The decrease of transpiration rate could reduce salt loading into the leaves and hence prolong the leaf lifespan by maintaining salts at subtoxic levels (Everard et al., 1994; Koyro, 2006). An aspect that deserves further comment is that salt-induced reduction in the transpiration rate was proportionally larger than those in the photosynthetic rates under extreme salinity conditions (300 and 400 mM NaCl) as explained by measuring of the net photosynthesis uptake (A) (Table 1), leading to improved photosynthetic water use efficiency (WUE). This result has already been reported for many halophytic species (Downton et al., 1985; Ayala and O'Leary, 1995). A priori the photosynthesis of *C. maritima* is affected under salt stress condition by an enhanced stomatal closure which leads to a substantial reduction of CO_2 diffusion to the carboxylation sites. 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 *C. maritima* of this study showed that repetitive salt shock led to decrease in the Chl(a and b) contents. NaCl-induced decrease in chlorophyll content has been widely reported (Koyro, 2006; Geissler et al., 2009) and it might be attributed to either a diminished chlorophyll biosynthesis due to nutrient deficiency or enhanced chlorophyllase activity (Ashraf and Bhatti, 2000). Our results indicated also a reduction in Chl(b) proportionally higher than that of Chl(a) and no significative change in the ratio Chl a/b with increasing salinity. Elisa et al. (2012) considers the reduction of Chl(b) content located in the light harvesting complex (LHC) is an adaptation in the LHC capacity, which in turn resulted in a reduction of the photosynthetic efficiency (Φ_c) and hence of oxidative stress (Moorthy and Kathiresan, 1999; Koyro, 2006).

Na^+ and Cl^- accumulation, the selectivity K^+/Na^+ and photosynthetic parameters directly influence the growth of plants. Our results confirm that *C. maritima* has a typical halophyte behaviour at 100 mM NaCl associated with a great capacity to accumulate Na^+ and Cl^- in the aerial parts and preserve the selectivity K^+/Na^+ . These results show a positive involvement of salt ions, in addition to K^+ in the optimum growth, favoring a suitable hydration and optimum operation of photosynthesis as has been demonstrated by measuring the uptake of the net CO_2 assimilation.

Salt adaption means that plants require, at least, some Na^+ to maintain their osmoticum for sustaining optimal uptake of water and nutrients and for proper metabolic functioning (Flowers et al., 1977). Such an adaption allows halophytic plants to save energy by utilizing Na^+ for osmotic balance, and thereby curtailing the requirement to synthesize osmolytes like proline and glycine betaine at least up to certain optimum NaCl concentration. This allows halophytes to allocate more energy and carbon resources towards the growth (Moseki and Buru, 2010). It has been reported that although the rate of salt uptake in succulent halophytes is high, the concentration of salt inside their cells remains low, due to the dilution effect (Greenway, 1968) and Na^+ compartmentalization as well as consequently maintaining the growth rate up to a certain optimum level. However at higher NaCl concentrations (eg. 400 mM), the growth is reduced. This reduction can be explained by the energy used for compartmentalization takes energy away from cell growth (Volkmar et al., 1998), decrease in photosynthesis, due to a stomatal limitation. However, *C. maritima* expresses pronounced selectivity K^+/Na^+ , regardless of the salinity at which the plant is exposed.

Salt-induced decrease the over reduction of the photosynthetic electron transport and hence the generation of reactive oxygen species (ROS) (Wang et al., 2003; Christian, 2005). The interaction of high Na^+ concentrations, as well as any other excessive cation concentrations, with the cell organelles leads to generated ROS.

Our results show that under repetitive salt shock (100 and 400 mM NaCl) in *C. maritima* is accompanied by high concentrations of H_2O_2 in roots might be linked to direct perception of salt stress. However, low level of H_2O_2 was observed in leaves and stems. So probably, the shoots of *C. maritima* could account for less-affected membrane permeability and better protection against oxidative stress under salt stress. MDA concentrations remained high in leaves and decrease in stems and roots. We did not observed significant differences between different concentrations of NaCl in different parts of the plant. Ellouzi et al. (2014) showed that MDA accumulated transiently in *C. maritima* after 24 h of salt shock. A transient increase in MDA was observed in the leaves of pea plants treated with NaCl for 8 h suggesting an adaptation of pea to salinity (Hernandez and Almansa, 2002). All the mentioned studies suggest that the salinity imposed would have produced oxidative stress without showing signs of oxidative damage. It is likely that MDA participates in the activation of early defense responses to salt stress by triggering relevant gene expression (Weber et al., 2004). It should be noted, however, that the role of MDA in the regulation of gene expression in plant responses to salt stress remains elusive.

Halophytic behaviour at 100 mM NaCl in *C. maritima* is accompanied by a significant increase of ascorbic acid in leaves and roots and slight increase in stems. It is likely that *C. maritima* responds to the high MDA levels by high regeneration of ascorbic acid. Ascorbic acid is involved in blocking of propagation of lipid peroxidation by recycling of tocopherol (Blokina et al., 2000). In the severe salinity

condition (400 mM NaCl) ascorbic acid significantly decreases compared to the plant that grows in 100 mM NaCl in leaves, and roots. In leaves of *C. maritima* under repetitive salt shock, a decrease of ascorbic acid is accompanied by an increase of MDA, and decrease in photosynthesis and chlorophyll content. These observations lead to oxidative damage related to the accumulation of MDA that necessitate vitamin C depletion.

As a whole, this study shows that the growth response of *C. maritima* under repetitive salt shocks is similar to what was reported under progressive salt stress. Neither salt shocks nor salt accumulation in aerial parts induces osmotic damage. Finally, the changes of malondialdehyde and ascorbic acid under salt shock revealed beneficial and damaging functions of these molecules at low and high salt shock, respectively.

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