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Implication of peroxisomes and mitochondria in the halophyte *Cakile maritima* tolerance to salinity stress

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Abstract

The role of mitochondria and peroxisomes in the tolerance of the halophyte *Cakile martima* to salt stress was studied. The plants were subjected to 0, 100, and 200 mM NaCl for 5 weeks. The evaluation of oxidative stress according to the content of malondialdehyde (MDA), carbonyl (CO^-) proteins, O_2^-, and H_2O_2, and the activities of several antioxidant enzymes, such as superoxide dismutase, peroxidase, and enzymes of the ascorbate-glutathione cycle were determined in two purified organelles, mitochondria and peroxisomes. The intact organelles were purified by centrifugation in *Percoll* density gradients. Results show that the content of MDA and CO^- proteins was higher in mitochondria than in peroxisomes under the salt stress. The antioxidant enzymes showed higher activities in peroxisomes than in mitochondria under different NaCl concentrations. These activities were highest at 100 mM NaCl. Our results suggest that the ascorbate glutathione cycle in peroxisomes plays a key role in the tolerance of *Cakile maritima* to salinity.

Additional key words: ascorbate-glutathione cycle, carbonyl proteins, H_2O_2, malondialdehyde, NaCl, O_2^-, peroxidase, superoxide dismutase.

Introduction

Sodium chloride is the most abundant salt in salinity-affected land. Salt stress is a major impairment to agricultural production that limits plant growth and yield. It is clear from the growing body of experimental evidence that salt stress affects the integrity of cellular membranes, the activities of enzymes, as well as the photosynthetic apparatus (Serrano *et al.* 1999). An important cause of this damage is the production of reactive oxygen species (ROS) (Smirnoff 1993). Chloroplasts, mitochondria, and peroxisomes are intracellular generators of ROS (Jimènez *et al.* 1998, Foyer and Noctor 2003, Del Río *et al.* 2006, Karkonen and Kuchitsu 2015). When ROS production is high, they can cause a substantial cellular damage (Sairam and Srivastava 2002, Ashraf 2009). The balance between ROS production and the activities of these ROS-removing systems determines the degree of possible oxidative stress. Thus, it determines to what extent signaling and/or damage will occur (Demidchik 2015). The question that arises here is how can a plant control and speed up the rate of ROS production and ROS scavenging when it is exposed to salinity. The amount of ROS depends on the rate of generation, reaction with other metabolites, such as proteins, lipids, and nucleic acids, the rate of their degradation, and the rate of their scavenging/neutralizing by enzymatic and/or non-enzymatic antioxidants (Mittler 2002, Ben Amor *et al.* 2005, Ashraf 2009). The plant cell and its organelles peroxisomes, chloroplasts, and mitochondria contain multiple enzymes or enzymes systems responsible for

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Abbreviations: APX - ascorbate peroxidase; BSA - bovine serum albumin; CAT - catalase; DHAR - dehydroascorbate reductase; GR - glutathione reductase; HPR - hydroxypyruvate reductase; MDA - malondialdehyde; MDHAR - monodehydroascorbate reductase; Mes - 2-(N-morpholino)ethanesulfonic acid; MOPS - 3-(N-morpholino)propanesulfonic acid; POD - non-specific peroxidase; PMSF - phenylmethylsulfonyl fluoride; ROS - reactive oxygen species, SOD - superoxide dismutase, Suc - sucrose.
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removing ROS (Asada 2000, Mittler 2002).

The components of the ascorbate glutathione cycle (the Halliwell-Asada cycle) represent a highly effective detoxification mechanism against H_2O_2 (Foyer *et al.* 1997). This cycle functions in most of the subcellular compartments of both photosynthetic and non-photosynthetic tissues (Del Río *et al.* 2002, Sevilla 2015).

Halophytes are known for their ability to quench ROS since they have a powerful antioxidant system (Ben Amor *et al.* 2005, 2006, Lokhande and Suprasanna 2012). However, only few information concerning NaCl-induced mitochondrial and peroxisomal ROS scavenging systems in detail do exist.

Cakile maritima (sea rocket) is an annual halophyte diploid species characterized by a C_3 pathway of photosynthesis. It has a short life cycle and produces a large number of seeds (10 000 seeds per plant) (Debez *et al.* 2012). This species is found frequently along the Tunisian seashore (Debez *et al.* 2004). In addition to its ecological interest for dune and saline soil stabilization, *C. maritima* has a potential economic significance as its seeds contain appreciable amounts of oil (40 % on dry mass basis) (Zarrouk *et al.* 2003). Ben Amor *et al.* (2006) showed that the relative salt tolerance of *Cakile maritima* is associated with high antioxidant enzyme activities. However, the implication of cell organelles for eliminating oxidative stress as well as their roles in salt tolerance is not known yet. Thus, the aim of this study was to investigate the relationship between response of *Cakile maritima* to NaCl-induced oxidative stress and its salinity tolerance and to examine the roles of mitochondria and peroxisomes in antioxidant defense.

Materials and methods

Plants and culture conditions: The mature seeds of *Cakile maritima* Scop. were collected from sandy beaches in Djerba, an island which is close to the Mediterranean seashore, 520 km south of Tunis. Seeds were sown in pots (4 seeds per pot) filled with 3 kg of inert sand and irrigated with distilled water until germination. After 3 weeks, seedlings were watered daily with a Hewitt (1966) nutrient solution (pH 7.3, electric conductivity, EC 2.7 mS cm^{-1}). Five-week-old seedlings were subjected to 0, 100, 200 mM NaCl for 5 weeks (the salt concentrations were daily stepwise increased with 50 mM NaCl). The experiment was conducted in a growth room under day/night temperatures of 25/15 °C and realtive humidities of 70/90 %, a 16-h photoperiod, and photosynthetic active radiation of 440 μmol m^{-2} s^{-1}.

Purification of cell organelles: Leaf mitochondria and peroxisomes were isolated by differential and density-gradient centrifugation as described by Jiménez *et al.* (1997) but with some minor modifications. All operations were realized at 0 °C. Leaves (50 g) were chopped in 200 cm^3 of extraction medium (pH 7.3) containing 30 mM 3-(N-morpholino)propanesulfonic acid (Mops), 1 mM EDTA, 0.35 M mannitol, 0.2 % (m/v) bovine serum albumine (BSA), and 0.1 M phenyl-methylsulfonyl fluoride (PMSF). The homogenate was filtered through two layers of *Miracloth*. The chloroplasts were sedimented by centrifugation of the homogenate at 2 200 *g* for 5 min. The supernatant was centrifuged at 12 000 *g* for 10 min, and the obtained supernatant was considered as the cytosolic fraction. The pellet was resuspended in a wash medium [10 mM Mops (pH 7.2), 0.35 M mannitol, 1 mM EDTA, and 0.1 % (m/v) BSA] and centrifuged at 2 200 *g* for 5 min. In the supernatant, mitochondria and peroxisomes were finally pelleted at 12 000 *g* for 15 min. The total enzyme activity in the organelle fraction was determined in the pellet that was resuspended in a small volume of the wash medium.

The separation of mitochondria and peroxisomes was achieved by a self-generated *Percoll* gradient. In fact, 2.5 cm^3 of the suspension of the "12 000 *g* pellet" in the wash medium was layered on a discontinuous gradient composed of 4 different concentrations of *Percoll* from the bottom to the top (53, 38, 20, and 15 %, v/v), respectively. Then, centrifugation at 12 000 *g* for 35 min was realized. We obtained purified peroxisomes and a fraction enriched with mitochondria. The intact peroxisomes were obtained definitively after a wash [10 mM Mops (pH 7.2), 0.35 M mannitol, and 1 mM EDTA] and a centrifugation at 12 500 *g* for 15 min. This step was repeated four times, and the pellet was delicately resuspended in a small volume of the same medium. On the other hand, 3 cm^3 of the extraction medium without PMSF was added in the fraction enriched with mitochondria, a centrifugation at 12 500 *g* for 15 min was made, 10 cm^3 of the wash medium was added to the pellet, and the same centrifugation as before was realized. The pellet was resuspended in 2.5 cm^3 of the wash medium and fractionated on a gradient composed at the bottom of 13 cm^3 of 30 % (v/v) *Percoll* in 0.42 M sucrose, 13.9 mM Mops (pH 7.2), and 0.14 % (m/v) BSA. The top contained 18.5 cm^3 of 28 % (v/v) *Percoll* in 0.41 M mannitol, 13.9 mM Mops (pH 7.2), and 0.14 % BSA. The gradients were centrifuged at 41 400 *g* for 35 min. The purified mitochondria were recuperated. The intact mitochondria were diluted 10 times with the wash medium without BSA and spun down at 12 000 *g* 4 times for 35 min. Finally, the pellet was carefully resuspended in a small volume of the same medium. In the studies of ascorbate peroxidase (APX) activity, an independent organelle isolation was used adding 20 mM sodium ascorbate to the extraction medium, and all of the other

solutions contained 2 mM ascorbate to prevent possible inactivation of APX.

Lipid peroxidation assay: The extent of lipid peroxidation in the different cell compartments was estimated by determining the concentrations of substances reacting with thiobarbituric acid (Buege and Aust 1972).

Protein carbonyl content assay: Carbonyl content was assayed by reaction with 2.4-dinitrophenylhydrazine as described by Levine *et al.* (1990) with some modifications. The homogenate [a mixture of 0.1 cm^3 of the mitochondrial suspension or peroxisomal suspension and a protease inhibitor (0.2 μg each of leupeptin and aprotinin)] reacted with 0.5 cm^3 of 10 mM 2.4-dinitrophenylhydrasine or 2 M HCl (control). The samples were then incubated at room temperature for 1 h with vortexing every 10 min, then the proteins were precipitated with trichoracetic acid. The tubes were centrifuged at 11 000 *g* for 3 min, and the supernatant was discarded. The pellet was washed with 1 cm^3 of an ethanol and ethyl acetate (1:1) mixture 3 times. The final protein pellets were dissolved in 0.5 cm^3 of guanidine (6 M, with 20 mM potassium phosphate adjusted to pH 2.3 with trifluoroacetic acid) at 37 °C for 1 min. The solution was centrifuged at 11 000 *g* for 3 min. Hydrochloric acid (2 M) was added into the supernatant instead of 2.4-dinitrophenylhydrazine as a blank. Absorbance was determined at 360 nm. The carbonyl content was calculated using a coefficient of absorbance (ε) of hydrazine (22 000 M^{-1} cm^{-1}).

Determination of H_2O_2 content: The H_2O_2 content was determined by peroxidase-coupled assay using 4-amino-antipyrine and phenol as donor substrates (Frew *et al.* 1983). Briefly, 0.15 cm^3 of each organelle fraction was mixed with 25 mM phenol, 5 mM 4-amino-antipyrine, 0.1 M potassium phosphate buffer (pH 6.9), 0.02 μM peroxidase, and 2.5 μM H_2O_2. Quinoneimine formation was measured at 505 nm.

Superoxide radical content in purified submitochondrial particles was determined according to the method of Boveris (1984) and following the superoxide dismutase-inhibitable oxidation of epinephrine using NADH and succinate as respiratory substrates. The amount of $O_2^{\cdot -}$ radicals produced was calculated using ε of 4.0 mM^{-1} cm^{-1} for epinephrine.

Enzyme assays: The activities of all enzymes were assayed in organelle samples diluted 2- to 8-fold with 50 mM potassium phosphate buffer (pH 7.8) containing 0.1 % (v/v) *Triton X-100*. Superoxide dismutase (EC 1.15.1.1) activity was assayed by the ferricytochrome *c* method using xanthine/xanthine oxidase as a source of superoxide radicals (McCord and Fridovich 1969). A reaction mixture consisted of 50 mM potassium phosphate buffer, 1 mM cytochrome *c*, 1 mM xanthine, 0.04 units of xanthine oxidase, and the crude enzyme extract. One unit of SOD was defined as the amount of SOD required to inhibit the rate of cytochrome *c* oxidation by 50 %.

Catalase (CAT; EC 1.11.1.6) activity was measured spectrophotometrically according to the method of Aebi (1984) by monitoring a decline in absorbance at 240 nm as H_2O_2 was consumed (ε = 39.4 mM^{-1} cm^{-1}). A reaction mixture contained 50 mM potassium phosphate buffer, 10 mM H_2O_2, and the crude enzyme extract.

Peroxidase (POD; EC 1.11.1.7) activity was determined spectrophotometrically by measuring the oxidation of *o*-dianisidine (3, 3'-dimethoxybenzidine) at 460 nm (Ranieri *et al.* 2000). One unit of the enzyme activity corresponds to an amount of oxidized dianisidine per minute.

Ascorbate peroxidase (APX; EC 1.11.1.11) activity was measured spectrophotometrically according to Jimènez et *al.* (1997) by following a decline in absorbance at 290 nm as ascorbate was oxidized (ε = 2.8 mM^{-1} cm^{-1}). The oxidation rate of ascorbate was estimated between 1 and 60 s after starting the reaction with the addition of H_2O_2.

Monodehydroascorbate reductase (MDHAR; EC 1.6.5.4) activity was assayed at 25 °C by monitoring a decrease in the absorbance at 340 nm (Arrigoni *et al.* 1981). Monodehydroascorbate was generated by the ascorbate/ascorbate oxidase system. A reaction mixture contained 100 mM potassium phosphate buffer (pH 7.0), 1 mM ascorbate, 0.2 mM NADH, 2 U of ascorbate oxidase from *Cucurbita* sp. (*Sigma*, St. Louis, USA), and the crude enzyme extract.

Dehydroascorbate reductase (DHAR; EC 1.8.5.1) activity was assayed following an increase in absorbance at 265 nm (Dalton *et al.* 1993) due to reduced glutathione dependent production of ascorbate and using the N_2-bubbled buffer. A reaction mixture (1.0 cm^3) contained 100 mM potassium phosphate buffer, 4 mM dehydroascorbate, 50 mM reduced glutathione, and the crude enzyme extract. The reaction rate was corrected for a non-enzymatic reduction of dehydroascorbate by reduced glutathione. A factor of 0.98 to account for a small contribution to the absorbance by oxidized glutathione was used.

Total glutathione reductase (GR; EC 1.6.4.2) activity was determined by following the rate of NADPH oxidation as measured by a decrease in absorbance at 340 nm (Edwards *et al.* 1990). Reactions were carried out in 100 mM Hepes (pH 7.8) containing 4 mM NADPH, 3 mM $MgCl_2$, 1 mM $EDTANa_2$, and 0.62 mM oxidized glutathione. The reaction rate was corrected for a small and non-enzymatic oxidation of NADPH by reduced glutathione.

D-glucose 6-phosphate: NADP 1-oxidoreductase (Glc-6-P; EC 1.1.1.49) was assayed spectrophoto-

metrically monitoring NADP reduction at 340 nm. A reaction mixture contained 50 mM Hepes-NaOH (pH 7.2), 5 mM $MgCl_2$, and 1 mM NADP, and was initiated with 1 mM glucose-6-phosphate (Doehlert *et al.* 1988).

Cytochrome *c* oxidase (EC 1.9.3.1) activity was based on cytochrome *c* oxidation and was followed at 550 nm (Hernández *et al*. 1993). A reaction mixture consisted of 100 mM potassium phosphate buffer, 0.4 mM sodium hydrosulfate, and 24 µM cytochrome *c*. Fumarase (EC 4.2.1.2) activity was estimated by the measurement of the increase of absorbance at 240 nm (Bergmeyer *et al.* 1974) in consequence to the formation of malate from fumarate. One unit corresponds to conversion of 1.0 µmol of L-malate to fumarate per minute at pH 7.6 and 25 ºC. Hydroxypyruvate reductase (HPR; EC 1.1.1.81) activity was assayed according to Kleczkowski and Edwards (1989). A reaction mixture contained 100 mM Mes (pH 6.5), 0.2 mM NAD(P)H, and 1 mM hydroxypyruvate.

Chlorophyll content was determined according to Torrecillas *et al.* (1984). The extraction was realised with acetone 80 % and the absorbance was measured at 649 and 665 nm. Protein content was estimated according to Bradford (1976).

Statistical analysis: Results were expressed as means ± standard errors. All analyses were carried out with *GraphPad Prism 6.0* for Windows (*GraphPad* software, San Diego, CA, USA). Significant differences between treatment effects were determined by one-way *ANOVA* followed by the Tukey post-hoc test for multiple comparisons.

Results

Cakile maritima requires 100 mM NaCl (Fig. 1*A*) to express its maximal growth rate (125 % of control). Plants exposed to 200 mM NaCl have shown a growth reduction of 13 % compared to control plants. However, no symptoms of leaf necrosis were shown (Fig. 1*B*).

The purity of isolated peroxisomes and mitochondria is illustrated in Tables 1 and 2. In mitochondria, on the basis of the specific activity of HPR and CAT, there was contamination of around 8.5 % with peroxisomes. Similarly, the mitochondrial contamination of peroxisomes estimated from the fumarase and cytochrome *c* oxidase activities was around 13 and 20 %, respectively. Glucose-6P dehydrogenase activity and chlorophyll content did not exceed 5 % in the two purified organelles.

Protein carbonyl content and lipid peroxidation (Fig. 2*A*,*B*) in mitochondria remained virtually independent of NaCl concentration. On the contrary, in peroxisomes, protein carbonyl content as well as malondialdehyde (MDA) content decreased by 20 and 30 %, respectively, at 100 mM NaCl in comparison to control plants. However, a slight increase of 5 % in protein carbonyl content and a decrease of 10 % in MDA content were observed at 200 mM NaCl, respectively. The content of H_2O_2 and $O_2^{\cdot-}$ (Fig. 2*C*,*D*) increased with NaCl concentration in mitochondria, but in peroxisomes, these ROS did not present a significant variation with salinity.

Activity of CAT (Fig. 3*A*) was much higher in peroxisomes than in mitochondria independently of salinity (12 times higher at 100 mM NaCl and 4 times higher at 200 mM NaCl in comparison to the mitochondria fraction). It revealed a maximum activity at 100 mM NaCl. In both mitochondrion fraction and peroxisome fraction, CAT activities were higher under salinity than in control plants.

Independently of salinity, SOD activity (Fig. 3*B*) was higher in peroxisomes than in mitochondria. In peroxisomes, SOD activity increased by 60 and 75 %, respectively, in plants treated with 100 and 200 mM NaCl compared to control plants. However, in the mitochondria fraction, SOD activity increased only by 40 % under 100 and 200 mM NaCl.

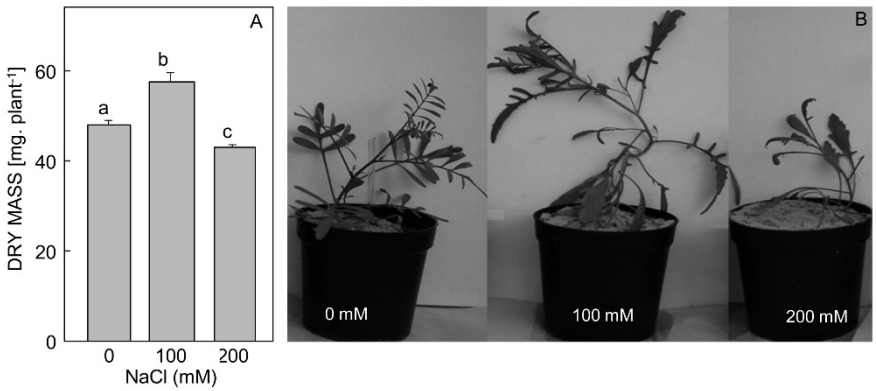

Fig. 1. Effects of NaCl on dry mass (*A*) and morphology (*B*) of five-week-old *Cakile maritima*. Means ± SE, $n = 10$, different letters indicate significant differences at $P < 0.05$ between treated and control plants.

Table 1. Specific activities [μmol mg^{-1}(protein) min^{-1}] of marker enzymes in mitochondria - fumarase and cytochrome *c* oxidase (CCO) and in peroxisomes - hydroxypyruvate reductase (HPR) and catalase (CAT) from five-week-old *Cakile maritima*. Means ± SEs, $n = 10$, percentage of total activity.

Organelles	Fumarase activity	%	CCO activity	%	HPR activity	%	CAT activity	%
Mitochondria	1.67 ± 0.016	87.4	2.81 ± 0.047	80	0.63 ± 0.04	8.3	0.29 ± 0.03	8.5
Peroxisomes	0.24 ± 0.048	12.6	0.70 ± 0.096	20	7.00±± 0.32	91.7	3.10 ± 0.08	91.5

Table 2. Specific activity [μmol mg^{-1}(protein) min^{-1}] of marker enzyme glucose-6-phosphate dehydrogenase (G6PDH) and chlorophyll content [μg cm^{-3}] in crude extracts from five- week-old *Cakile maritima*. Means ± SEs, $n = 10$, percentage of total content or activity.

Organelles	Chlorophyll content	%	G6PDH activity	%
Mitochondria	0.04 ± 0.001	4.3	0.09 ± 0.001	3.6
Peroxisomes	0.039 ± 0.007	4.2	0.11 ± 0.005	4.4
Chloroplasts	0.840 ± 0.040	91.5	2.30 ± 0.016	9.0

Activity of POD (Fig. 3*C*) was almost the same in different organelles of control plants. However, the impact of salinity was much more pronounced in the mitochondria fraction. In fact, POD activity increased 4 times at 100 mM NaCl and 6.5 times at 200 mM NaCl in comparison to control plants. In peroxisomes, POD activity increased only slightly under the salinity.

Activity of APX (Fig. 4*A*) was 2.5 times higher in peroxisomes than in mitochondria independently of salinity. It increased by 20 % in plants treated with 100 mM NaCl and decreased by 5 % under 200 mM NaCl in comparison to control plants. However, APX activity in mitochondria was rather low and independent on salinity.

Under different NaCl concentrations, GR activity (Fig. 4*B*) was more important in peroxisomes than in mitochondria. Generally, GR activity, increased by 10 % at the moderate salinity and decreased by 4 % at the high salinity in both the organelles. Similarly, MDHAR and DHAR activities (Fig. 4*C*,*D*) were higher in peroxisomes than in mitochondria. These activities changed insignificantly under salinity with exception of a slight increase in peroxisomes under 1 mM NaCl.

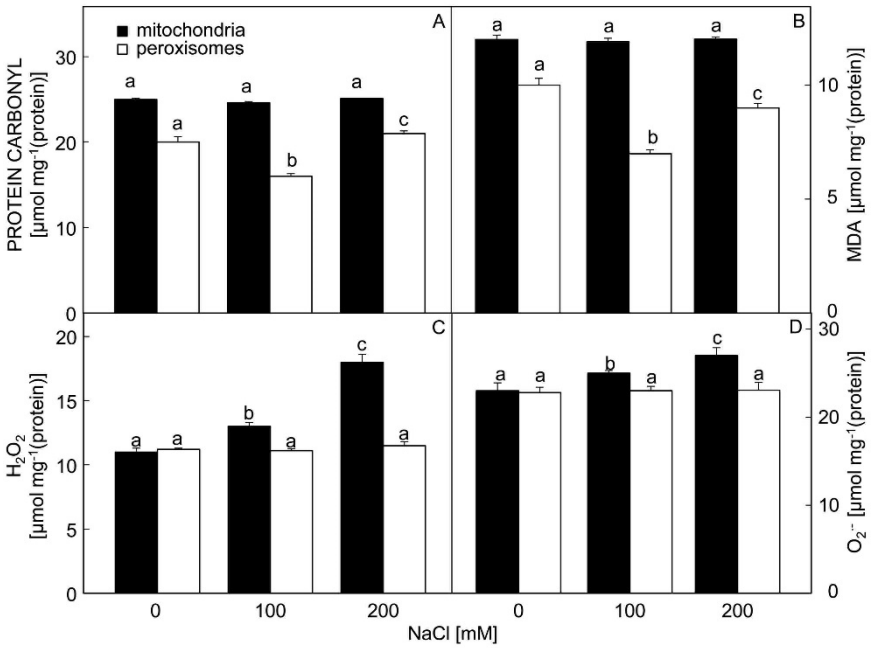

Fig. 2. Effects of NaCl on protein carbonyl content (*A*), malonyldialdehyde (MDA) content (*B*), hydrogen peroxide content (*C*), and superoxide radical content (*D*) in mitochondria and peroxisomes of five-wee-old *Cakile maritima*. Means ± SEs, $n = 3$, different letters indicate significant differences at $P < 0.05$ between treated and control plants.

Discussion

Cakile maritima is considered to be a halophyte plant, and in fact, its maximum growth was observed under a moderate salinity. The relative salt tolerance of *Cakile maritima* has been described to be associated with an efficient antioxidant system including glutathione content together with a low MDA content, electrolyte leakage, and H_2O_2 content (Ben Amor *et al.* 2006, 2010). Differences in antioxidant enzymes and non-enzymatic antioxidants between individual cell organelles in glycophytes, such as *Pisum sativum*, (Jiménez *et al.* 1997, 1998, Gómez *et al.* 2003), *Solanum lycopersicum* (Kuzniak and Skłodowska 2005), and *Arabidopsis thaliana* (Rodríguez-Serrano 2009), have been previously reported. However, to our knowledge, there is no information concerning the implication of the organelles in the antioxidant defense as well as the tolerance to salinity in *Cakile maritima*. The question posed here is how halophytes in general and *Cakile maritima* specifically, control the rate of ROS production and ROS scavenging when they are exposed to salinity. Our results reveal that in *Cakile maritima*, mitochondria and peroxisomes behaved differently under salinity. These differences appeared at two levels: oxidative stress parameters and antioxidant enzymes. Our results indicate that salinity induced a more severe oxidative stress in mitochondria than in peroxisomes where MDA and CO^- proteins content remained generally unchanged.

In the present study, two hypotheses were suggested. The first hypothesis considers that the majority of ROS are essentially produced in mitochondria and not in peroxisomes under salt stress. Consequently, the antioxidant enzymes might be unable to ensure ROS detoxification in mitochondria. The second hypothesis considers that the production of ROS under salt stress occurred not only in mitochondria but also in peroxisomes. Nevertheless, the efficient antioxidant defense is in the peroxisomes. Our results show an important content of H_2O_2 and O_2^- in both peroxisomes and mitochondria, which excludes the first hypothesis. However, H_2O_2 and O_2^- content increased with the increased salinity in mitochondria but not in peroxisomes. These results are in agreement with those of Mittova *et al.* (2004) who showed that the increase of oxidative stress was detected essentially in mitochondria and not in peroxisomes. This suggests the presence of efficient antioxidant enzymes in peroxisomes.

The SOD is one of the most important enzymes of the plant defense system (Del Río *et al.* 1992). Our study has revealed that at moderate and high salinities, the dismutation of superoxide by SOD was realized essentially in peroxisomes. It has been suggested previously, that peroxisomes are organelles with the capacity to generate and release $O_2^{\bullet-}$ and H_2O_2 into the cytosol, which can contribute to communication among cell compartments (Del Río *et al.* 2006, Gill and Tuteja 2010, Corpas *et al.* 2015). Further, an increase in pea mitochondrial Mn-SOD with salinity was found by Gómez *et al.* (1999) and a correlation between salt tolerance and increased Mn-SOD activity in tomato, wheat, and pea (Hernández *et al.* 1993, Sairam and Srivastava 2002, Mittova *et al.* 2003). However, a discrepancy exists for the peroxisomal Mn-SOD isoform since it is not induced under salinity in pea tolerant or sensitive cultivars (Corpas *et al.* 1993b).

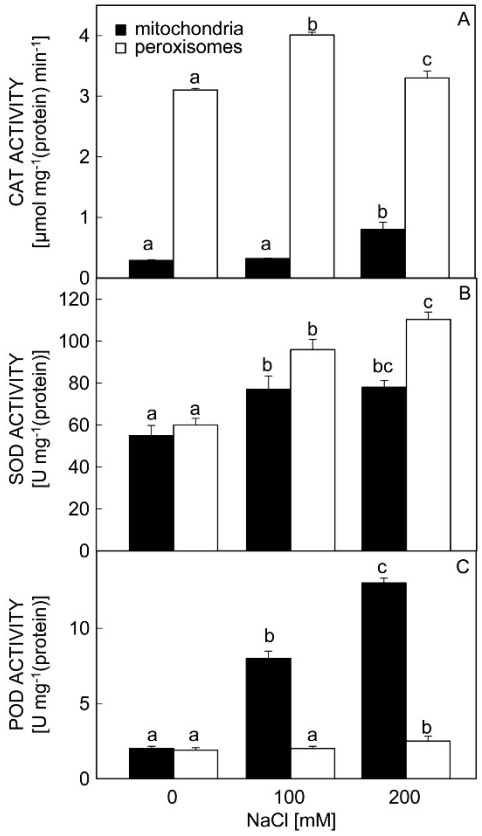

Fig. 3. Changes in catalase (CAT) (*A*), superoxide dismutase (SOD) (*B*), and peroxidase (POD) (*C*) activities in mitochondria and peroxisomes from five-week-old *Cakile maritima* in response to NaCl. Means ± SEs, $n = 3$, different letters indicate significant differences at $P < 0.05$ between treated and control plants.

The H_2O_2 formed after dismutation of $O_2^{\bullet-}$ in peroxisomes of *Cakile maritima* under salt stress was rapidly eliminated by several antioxidant enzymes. However, in mitochondria, H_2O_2 content remained high under the salinity, which implicates a less efficient antioxidant system in this organelle. The H_2O_2 produced in peroxisomes is mostly decomposed by CAT isoenzymes localized in the matrix usually in the form of organized structures called crystalline cores (Igamberdiev and Lea 2002). The present study has shown an important activity of CAT in peroxisomes essentially at the

moderate NaCl concentration suggesting a key role for this enzyme in *Cakile maritima* salt tolerance. However, a decline in CAT activity was described in pea peroxisomes under salinity (Corpas *et al.* 1993b). On the other hand, in a previous study (Ben Amor *et al.* 2006), we showed a decline in CAT activity under salinity in the leaves of *Cakile maritima*. These data indicate that apart from CAT, the presence of other antioxidant enzymes localized in peroxisomes is probably involved in the elimination of H_2O_2. There are several experimental evidences for the existence of the ascorbate-glutathione cycle in peroxisomes (Jiménez *et al.* 1997, Mittova *et al.* 2000, Del Rio *et al.* 2002, Ashraf 2009, Hu *et al.* 2012). Our results indicate that APX, MDHAR, DHAR, and GR played a crucial roles in the elimination of oxidative stress and therefore in the tolerance of *Cakile maritima* especially under salt stress. These enzymes presented mostly higher activities at 100 mM NaCl than at 0 and 200 mM NaCl which is similar to results previously described by Gómez *et al.* (1999) for pea mitochondria. However, these activities were higher in peroxisome than in mitochondria in different salt concentrations. This suggests the essential role of peroxisomes for salt tolerance of this halophyte. It is worthy to note that scavenging H_2O_2 is carried out not only by CAT but also by the ascorbate glutathione cycle. On the other hand, several studies showed the implication of chloroplasts in the detoxification of ROS (Asada 1999, Foyer and Noctor 2003). There is a possibility that chloroplasts of *Cakile maritima* could also play a role in the elimination of ROS and in the tolerance to salinity.

The current study has shown the important role of peroxisomes for the antioxidant defense against oxidative stress and consequently their implication on the tolerance of *Cakile maritima* under salt stress.

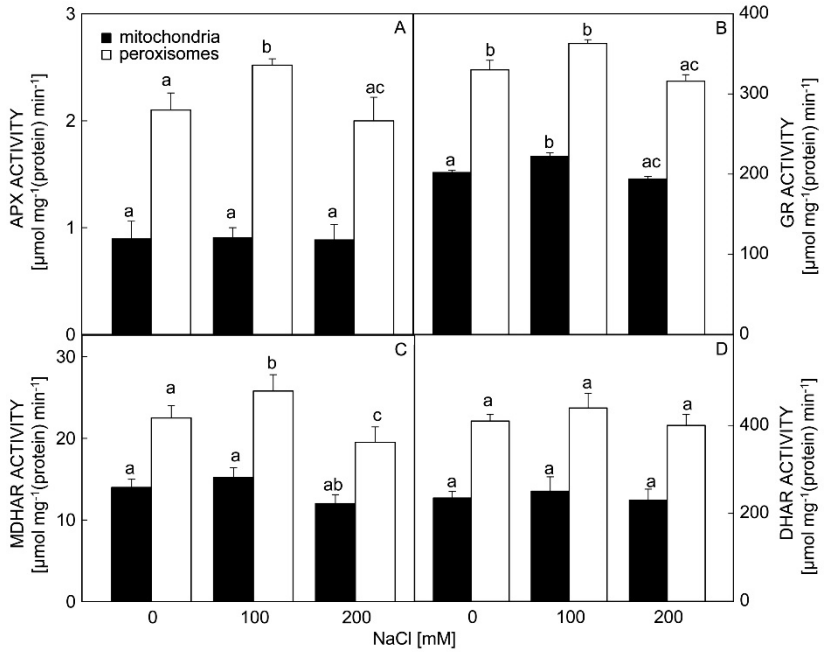

Fig. 4. Changes in activities of ascorbate peroxidase (APX) (*A*), glutathione reductase (GR) (*B*), monodehydroascorbate reductase (MDHAR) (*C*), and dehydroascorbate reductase (DHAR) (*D*) in mitochondria and peroxisomes from five-week-old *Cakile maritima* in response to NaCl. Means ± SEs, $n = 3$, different letters indicate significant differences at $P < 0.05$ between treated and control plants.

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