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Antioxidant Enzyme Activities as a Tool to Discriminate Ecotypes of *Crithmum maritimum* L. Differing in Their Capacity to Withstand Salinity

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1. Introduction

Salt stress causes a range of adverse effects in plants, mainly ionic disorders, osmotic stress and nutritional imbalance. A common feature of these effects is the production of reactive oxygen species (ROS) (Ashraf and Foolad, 2007). Thus, salt stress causes stomatal closure, which reduces the CO_2/O_2 ratio inside leaf tissues and inhibits CO_2 fixation (Hernández *et al.,* 1999). As a consequence, an over reduction of the photosynthetic electron transport chain occurs, which causes the generation of ROS such as singlet oxygen ($^1O_2$), superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical (•OH). It is now widely accepted that ROS are responsible for various stress-induced damages to macromolecules and ultimately to cellular structure (Mittler, 2000; Ashraf, 2009). Plants are equipped with a set of non-enzymatic scavengers and of antioxidant enzymes that act in concert to alleviate cellular damage under oxidative stress conditions (Foyer and Noctor, 2000). Superoxide dismutase (SOD) reacts with the superoxide radical at almost diffusion-limited rates to produce H_2O_2 (Scandalios, 1993). H_2O_2 is scavenged by peroxidases, especially ascorbate peroxidase (APX), and catalase (CAT). CAT has been found predominantly in leaf peroxisomes where it functions chiefly to remove H_2O_2 formed in photorespiration or in β-oxidation of fatty acids in the glyoxysomes (Dat *et al.*, 2000). APX, which uses ascorbic acid as a reductant in the first step of the ascorbate-glutathione cycle is the most important plant peroxidase involved in H_2O_2 detoxification (Foyer and Halliwell, 1976; Noctor and Foyer, 1998). ROS are also scavenged non-enzymatically by hydrophilic antioxidants, such as ascorbate and glutathione (GSH), most of them being found in photosynthetic tissues.

In the last two decades, a number of reviews have concentrated much on the role of various antioxidant enzymes and metabolites in plant salt tolerance (Jitesh *et al.*, 2006; Ashraf, 2009). Due to considerable variations in the mechanisms of defense against ROS among plant species, it is difficult to generalize the involvement of this phenomenon in salt tolerance

(Ashraf and Harris, 2004). A number of experimental approaches have been adopted to understand the link between salt tolerance and antioxidant activities. Several studies compared crop species, genera and cultivars differing in salt tolerance, and showed considerable variations in the production of antioxidant in response to salt stress. Moreover, increased levels of antioxidant enzymes and/or metabolites have been correlated to the degree of salt tolerance in a number of plant species including wheat, rice, maize, cotton, tomato and potato (Ashraf, 2009). However, such relationship could not be found in other plants such as cowpea (Cavalcanti *et al*., 2004), *Arabidopsis* (Katsuhara *et al*., 2005) and strawberry (Turhan *et al*., 2008).

Recently, many authors addressed investigations on naturally salt tolerant plants (or halophytes) in which the mechanisms of salt tolerance are fully developed and functional to make them productive under stress. Jithesh *et al.* (2006) reviewed the regulation of antioxidant enzymes under salt stress in halophytes, especially those of mangroves. They concluded that antioxidant enzymes protect halophytes from deleterious ROS production during salt stress. The antioxidant response in halophytes, like in glycophytes, varies among species and genera. Most studies reported an induction in the activities of antioxidant enzymes and an accumulation of antioxidant metabolites, in response to salinity (Cherian *et al*., 1999; Takemura *et al*., 2000; Parida *et al*., 2004). Moreover, some halophytes like *Thelungiella halophila* exhibited high levels of SOD expression even in unstressed conditions (Taji *et al*., 2004). Thus, the SOD activities in mangrove *Rhizophora stylosa* were more than 40 times higher than those of the glycophyte pea. High concentration of ascorbate was also observed in the leaves of that plant. This robust antioxidant activity is considered to be effective in protecting mangroves from excess irradiance (which generates ROS) under natural conditions (Cheeseman *et al*., 1997).

Higher antioxidant defences in leaves compared to those of roots accounted for the survival of the halophyte *Crithmum maritimum* L. under high salinity conditions (Ben Hamed *et al*., 2007). In the same species, Ben Amor *et al*. (2005) showed an increased total SOD activity in shoot tissues in presence of 50 mM NaCl stress while SOD activity in root tissues decreased upon salt treatment (up to 200 mM). These findings showed that the activities of antioxidant enzymes under salt stress may vary depending on the tissues. It may also depend on the conditions of treatments and on the species from which ecotypes were collected. In the present work, we examined the effects of salt stress on two ecotypes of *Crithmum maritimum* grown under the same controlled conditions. The effects of salt stress were investigated on growth, lipid peroxidation, electrolyte leakage, hydrogen peroxide levels and antioxidative enzyme activity. The differential response of that system might contribute to better understand the mechanism of salinity resistance and, in turn, might lead to the development of elite ecotypes able to withstand high salinity levels in the soils.

2. Material and methods

2.1 Plant material and growth conditions

Seeds of two *Crithmum maritimum* (Apiaceae) ecotypes were collected from coastal sites near Tabarka (North-West of Tunisia, humid bioclimatic stage) and Korbous (North-East of Tunisia, semi-arid bioclimatic stage). Seeds were sown in pots (4 seeds per pot) filled with 3 kg of inert sand and irrigated with distilled water until germination. Then, seedlings were daily watered with Hewitt solution (Ben Hamed *et al.,* 2007). Thirty four day-old seedlings were partitioned into 3 lots, which were treated with 0, 100, and 300 mM NaCl, respectively

(the salt concentrations were daily stepwise increased with 50 mM NaCl). The experiment was conducted in a growth room under controlled conditions : 15-25°C temperature and 70-90% relative humidity, 8-16 h night-day photoperiod, and 440 µmol m^{-2} s^{-1} photosynthetic active radiations (PAR). Plants were harvested at the beginning of salt treatment (initial harvest) and after 50 days. For plant growth, plants were separated into leaves, stems and roots. Samples from fully expanded leaves were liquid N_2 frozen and stored at -80 °C for enzyme activity analysis.

2.2 Plant growth

Plant shoot and root dry weights (DW) were measured after 96 h at 80°C. The relative growth rate (RGR) was also calculated.

$$RGR\ (day^{-1}) = M/\underline{M}\ \Delta t$$

Where Δ is the difference between the values at the final and initial harvests, *t* the salt treatment duration (days), and $\underline{M}$ the logarithmic mean of M, the whole plant dry weight (g):

$$\underline{M} = \Delta M/\Delta \ln M)$$

2.3 H_2O_2 determination

H_2O_2 was extracted by homogenizing plant leaves with phosphate buffer (50 mM, pH 6.8) including 1 mM hydroxylamine. The homogenate was centrifuged at 6000 *g* for 25 min. The H_2O_2 concentration in the supernatant was then measured colorimetrically (Chaparzadeh *et al.*, 2004). Aliquot of the extract was mixed with 0.1% titanium chloride in 20% (v/v) H_2SO_4 and the mixture was centrifuged at 6000 *g* for 15 min. The intensity of yellow color of the supernatant was measured at 410 nm. H_2O_2 concentration was calculated using the extinction coefficient 0.25 $mM^{-1}cm^{-1}$, and expressed on the basis of fresh weight (FW).

2.4 Lipid peroxidation

The extent of lipid peroxidation was estimated by determining the concentration of malondialdehyde (MDA) (Hagege *et al.*, 1990). Leaf materials were homogenized in 0.1% (w/v) TCA solution. The homogenate was centrifuged at 15000 *g* for 10 min and 1 ml of the supernatant obtained was added to 4 ml of 0.5% (w/v) TBA in 20% (w/v) TCA. The mixture was incubated at 90°C for 30 min, and the reaction was stopped by placing the reaction tubes in an ice water bath. Then, the samples were centrifuged at 10000 *g* for 5 min, and the absorbance of the supernatant was read at 532 nm. The value for non-specific absorption at 600 nm was subtracted. The concentration of MDA was calculated from the extinction coefficient 155 $mM^{-1}cm^{-1}$.

2.5 Electrolyte leakage

Fresh leaf samples were cut into discs of uniform size (5 mm diameter) and placed in test tubes containing 10 ml of double distilled water. The tubes were incubated in a water bath at 32°C for 2 h and the initial electrical conductivity of the medium (EC1) was measured. Samples were heated at 100°C for 20 min to release all electrolytes, cooled to 25°C and the final electrical conductivity (EC2) was measured (Sairam *et al.*, 2002). The electrolyte leakage (EL), expressed in % of total electrolytes, was calculated by using the formula: EL= (EC1/ EC2) x 100.

2.6 Enzyme extractions

All of the following operations were performed at 4°C. Fresh leaf and root samples (0.5 g) were rapidly extracted in a pre-chilled mortar with 10% (w/v) PVP (polyvinylpyrrolidone) in 50 mM K-phosphate buffer (pH 8), containing 0.1 mM EDTA (ethylenediaminetetraacetic acid), 1 mM DTT (dithiothreitol), and 0.5 mM PMSF (phenyl-methyl-sulphonyl-fluoride). For ascorbate peroxidase extraction, 20 mM sodium ascorbate was added to the medium to maintain the enzyme active during the extraction procedure. The homogenate was centrifuged at 12000 *g* for 30 min. and the supernatant was dialysed for 2 h against the extraction buffer containing only 5 mM sodium ascorbate. Three replicates per treatment were used. The supernatants were collected and protein concentration was determined according to Bradford (1976), using bovine serum albumin as a standard.

2.7 Enzyme assays

Total superoxide dismutase (SOD, EC 1.11.1.5) activity was assayed according to Scebba *et al.* (1999). Increasing volumes (5, 10, 20, and 40 µl) of leaf and root crude extracts were added to the reaction mixture at a final volume of 3 ml. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.8), 0.1 mM EDTA, 13 mM L-methionine, 2 µM riboflavin and 75 µM NBT (nitroblue tetrazolium). The reaction was started by exposing the mixture to cool white fluorescent light for 15 min. The blue colour developed was measured spectrophotometrically at 560 nm. The volume of sample causing 50% inhibition of colour development (compared to the blank where sample was replaced by extraction buffer) contained one unit of SOD activity and the specific activity of plant extracts was expressed as units per mg of protein.

Total catalase (CAT, EC 1.11.1.6) activity was measured spectrophotometrically according to the method of Lück (1965), by monitoring the decline in absorbance at 240 nm due to H_2O_2 consumption. The 3 ml reaction mixture contained 66 mM sodium phosphate buffer (pH 7.0) and 30% H_2O_2 (the absorbance should be about 0.5 at 240 nm and with a 1 cm light path). The reaction was initiated by adding an appropriate dilution of the shoot or root crude extract to this solution. CAT activity was expressed as units per mg of protein, one unit being the amount of enzyme which liberates half the peroxide in 100 s at 25°C (Lück, 1965).

Total 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). The reaction mixture contained 20 mM phosphate buffer (pH 5.0), 1 mM dianisidine, 3 mM H_2O_2 and 50 µl of extract. POD specific activity was expressed as units (µmol of dianisidine oxidized per minute) per mg of protein.

Total ascorbate peroxidase (APX, EC 1.11.1.11) activity was measured spectrophotometrically according to Nakano and Asada (1981) by following the decline in absorbance at 290 nm due to ascorbate oxidation. The oxidation rate of ascorbate was estimated between 1 and 60 s after starting the reaction with the addition of H_2O_2. The 1 ml reaction mixture contained 50 mM HEPES-NaOH (pH 7.6), 0.22 mM ascorbate, 1 mM H_2O_2, and an aliquot of enzyme extract. Corrections were made for the low, non-enzymatic oxidation of ascorbate by H_2O_2 and for the oxidation of ascorbate in the absence of H_2O_2. APX activity was expressed as units (µmol of ascorbate oxidized per minute) per mg of protein.

Total glutathione reductase (GR, EC 1.6.4.2) activity was determined by following the rate of GSSG-dependent oxidation of NADPH, through the decrease in the absorbance at 340 nm (Di Baccio *et al.*, 2004). The assay mixture (1 ml final volume) was composed of 0.4 M

potassium phosphate buffer (pH 7.5), 0.4 mM Na_2EDTA, 5.0 mM GSSG and 100 µl of crude extract. The reaction was initiated by the addition of 2.0 mM NADPH. Corrections were made for the background absorbance at 340 nm, without NADPH. Activity was expressed as units (µmol of NADPH oxidized per minute) per mg of protein.

2.8 Native gel electrophoresis and SOD activity staining

Samples of crude *C. maritimum* leaf extracts were analyzed by electrophoresis in 10% (w/v) polyacrylamide slab gel, at pH 8.9 under non-denaturating conditions.
Staining for SOD activity was carried out as described by Beauchamp and Fridovich (1971). The gel was first soaked in 50 mM sodium phosphate, (pH 7.5) containing 4.8 mM 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) in darkness for 20 min, followed by soaking in 50 mM sodium phosphate (pH 7.5) containing 0.4% (v/v) N, N, N', N'-tetramethylethylenediamine (TEMED) and 26 µM riboflavin, and subsequently illuminated under white light for 10 min. The three types of SOD, namely Fe-SOD, Mn-SOD and Cu-Zn SOD, were identified using inhibitors: Mn-SOD was visualised by its insensitivity to 5 mM H_2O_2 and 2 mM KCN, while Cu-Zn SOD was sensitive to 2 mM KCN and Fe-SOD was inhibited by 5 mM H_2O_2 (Navari-Izzo *et al.*, 1998).

2.9 Statistical analysis

Analysis of variance (ANOVA) using AV1W MSUSTAT program with orthogonal contrasts and mean comparison procedures was performed to detect significant differences between treatments. Mean separation procedures were carried out using the multiple range tests with Student's least significant difference (LSD) ($P \leq 0.05$).

3. Plant growth parameters

Growth of both ecotypes was estimated through dry mass production and RGR. The response of Tabarka plants to salinity was different to that of Korbous ecotype: whereas growth decreased under low (100 mM) and significantly under high (300 mM NaCl) salinity in Korbous plants (Fig. 1A and B), it increased by 40% under 100 mM NaCl in Tabarka plants.
In the following sections, we will try to check if the different physiological behavior of the ecotypes of *C. maritimum* observed mainly under low salinity (100 mM NaCl) is concomitant with different or similar antioxidant responses.

3.1 Oxidative stress parameters

Lipid peroxidation, as estimated by the MDA content, electrolyte leakage and H_2O_2 content are considered as the most useful indicators to detect oxidative disturbances in plants. In Korbous ecotype, MDA level (Fig. 2A), H_2O_2 concentration (Fig. 2B) and electrolyte leakage (Fig. 2C) increased in the leaves of salt treated plants. Conversely, all these parameters were higher in control plants of Tabarka ecotype.

3.2 Activity of antioxidant enzymes

Superoxide dismutase (SOD) is the first enzyme that eliminates superoxide radicals in plant cells. Total SOD activity in Korbous plants was unaffected by NaCl exposure but it increased in the leaves of salt-treated Tabarka ecotype (Fig. 3A). Individual activities of SOD

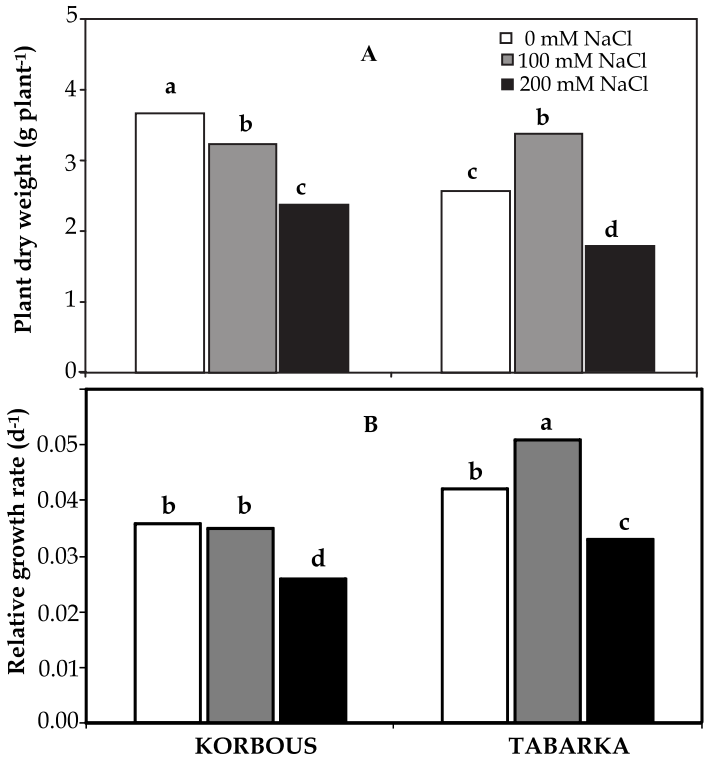

Fig. 1. Effect of NaCl on plant dry weight (**A**) and relative growth rate (**B**) of *Crithmum maritimum* ecotypes. The results are the means of 10 replicates. Means followed by different letters are significantly different at $P \leq 0.05$ according to student's LSD test.

isoforms in response to NaCl was examined after polyacrylamide gel electrophoresis (Fig. 3 B). The gel showed that plants from Korbous subjected to salt maintained steady activities of Mn and Fe SOD isoforms. Conversely, each isoform was stimulated by NaCl treatment in Tabarka ecotype and a new isoform (type Cu-Zn) was induced. Cu Zn, Mn and Fe SOD isoforms were identified in the presence of specific inhibitors (Figure 3 C).

SOD reaction on $O_2^{-\cdot}$ results in the production of H_2O_2, which needs to be controlled. The enzyme that are well known to play major roles in the detoxification of H_2O_2 are catalase (CAT), dianisidine (POD) and ascorbate peroxidases (APX) and Glutathione reductase (GR). In the absence of salt, the CAT activity in Tabarka plant was significantly higher than that of Korbous (Fig. 4A). The CAT activity did not change in plants from Korbous treated by 100 mM, while it increased in salt treated plants from Tabarka. POD activities increased under salinity conditions compared to control plants in both ecotypes (Fig. 4B). Also, we noticed higher control POD activities in Korbous ecotype. The activity of APX was significantly higher in Tabarka, both in the absence and presence of NaCl (Fig. 4C). Salt treatment did not affect enzyme activities in the two ecotypes. Under control conditions, the activity of GR was 50% higher in Korbous than in Tabarka plants (Fig. 4D). Salt-treated plants from Korbous exhibited a slight (-10%) decrease in GR activity, while a strong increase (+80%) in GR activity was observed in Tabarka ecotype.

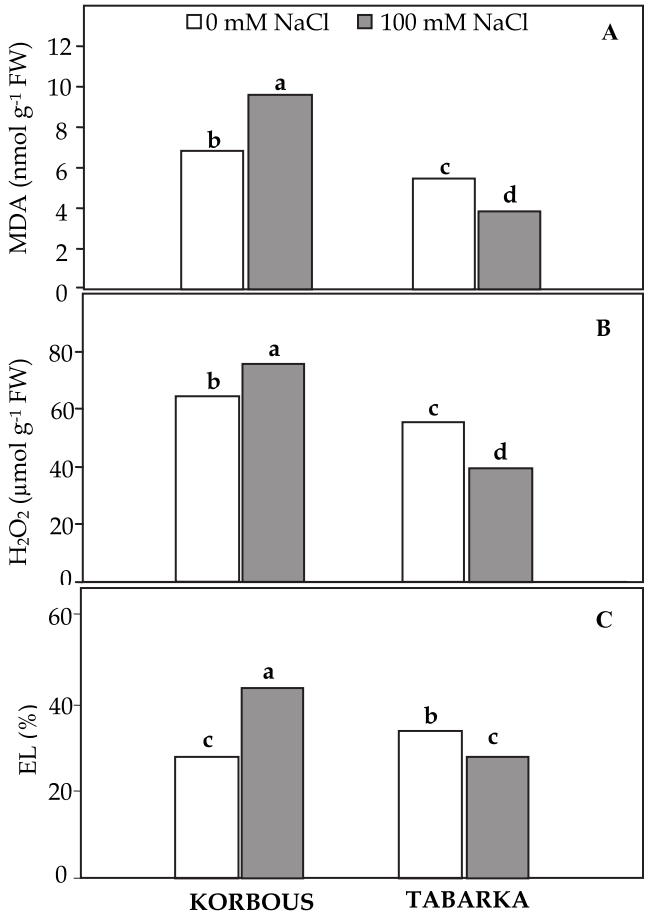

Fig. 2. Effect of NaCl on malondialdehyde levels (A), hydrogen peroxide (H_2O_2) concentration (B), and % of electrolyte leakage (C) in leaves of *Crithmum maritimum* ecotypes. The results are the means of 5 replicates. Means followed by different letters are significantly different at $P \leq 0.05$ according to student's LSD test.

4. Discussion

The two ecotypes of *Crithmum maritimum* exhibited strongly divergent responses to salinity. Tabarka ecotype showed an halotolerant behavior, as shown by stimulation of leaf biomass production and leaf expansion under 100 mM NaCl. Conversely, leaf growth and expansion of Korbous ecotype were significantly reduced under salt treatment, even at low NaCl concentration. These results showed an intraspecific variability in the response to salt stress of *C. maritimum*, a perennial Apiaceae, which is considered as a promising crop species for oil production (Zarrouk *et al.*, 2004). The same conclusion was drawn for another oilseed halophyte, *Cakile maritima* (Ben Amor *et al.*, 2006; Ksouri *et al.*, 2007; Megdiche *et al.*, 2009).

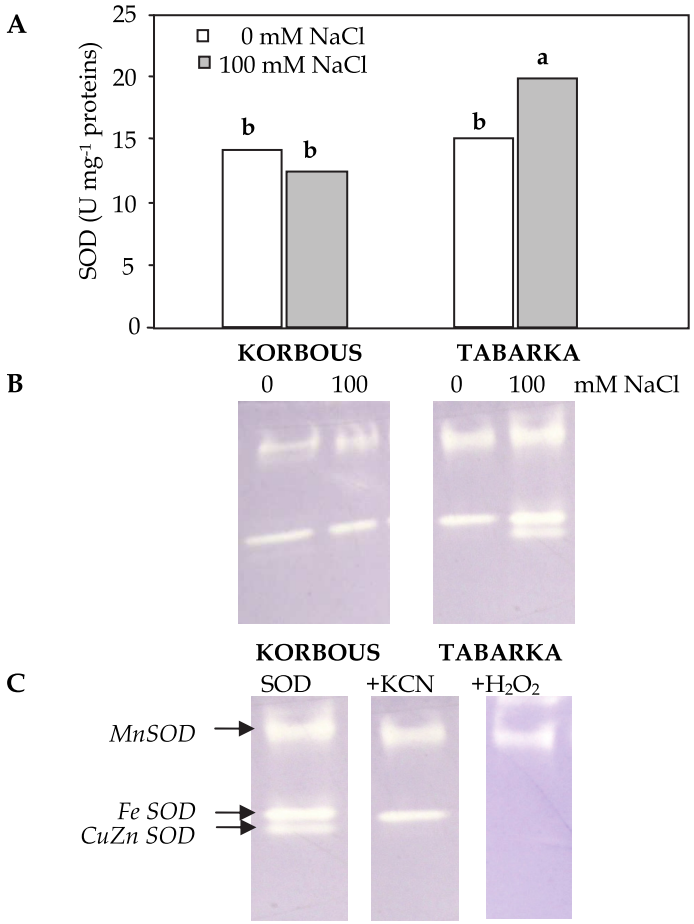

Fig. 3. Effect of NaCl on the total activity of superoxide dismutases (**A**) and in gel activities of SOD isoforms (**B**). Differentiation of CuZn, Mn and Fe SOD in the crude extract of leaves of salt-treated plants of *C. maritimum* (ecotype Tabarka) (**C**).The results in (A) are the means of 5 replicates. Means followed by different letters are significantly different at $P \leq 0.05$ according to student's LSD test.

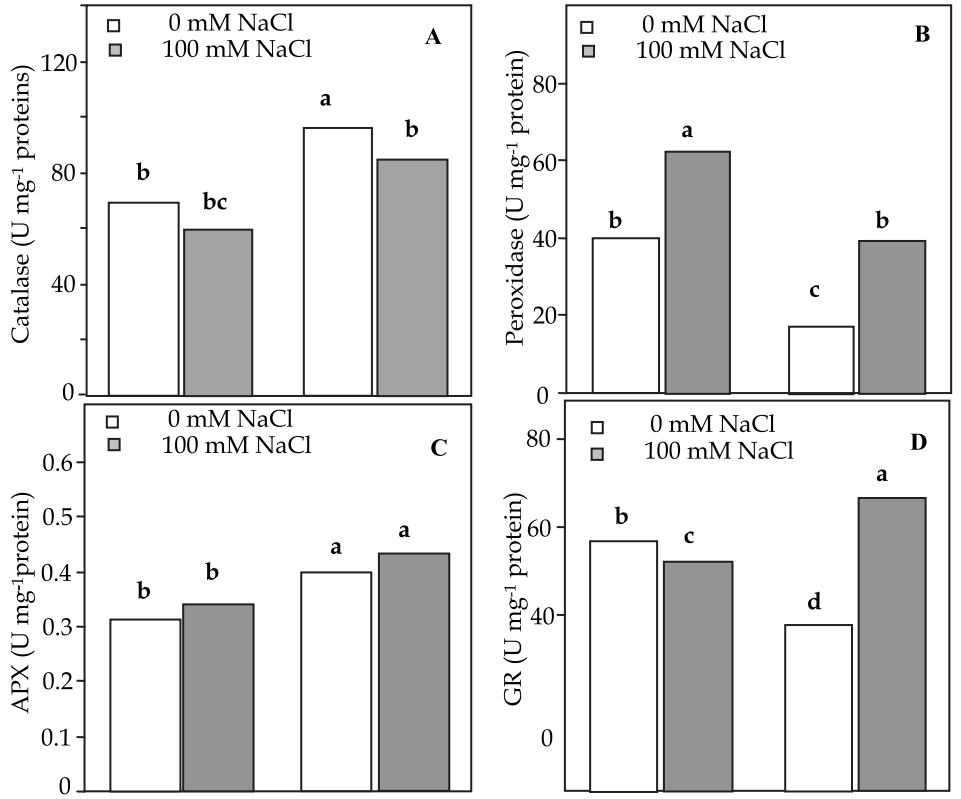

Fig. 4. Effect of NaCl on the total activity of catalase (CAT), dianisidine peroxidase (POD), ascorbate peroxidase (APX) and glutathione reductase (GR). Total CAT (**A**). POD (**B**). APX (**C**). GR (**D**). The results are the means of 5 replicates. Means followed by different letters are significantly different at $P \leq 0.05$ according to student's LSD test.

Salt stress is usually accompanied by the formation of various reactive oxygen species (ROS). These are toxic and may result in a variety of metabolic disturbances including photosynthetic component damages, protein and enzyme inactivation, and membrane permeabilization because of lipid peroxidation (Price and Hendry, 1991; Mittler, 2002; Meloni *et al.*, 2003). MDA level, H_2O_2 concentration and electrolyte leakage are considered as reliable indicators of the oxidative stress resulting from several abiotic constraints (Shulavev and Oliver, 2006). Our results showed variability of these indicators in leaves of the two ecotypes exposed to salt stress. The significant increases in MDA and H_2O_2 concentration and electrolyte leakage in Korbous leaves at 100 mM NaCl, compared to the lower MDA and H_2O_2 concentrations and electrolyte leakage in Tabarka leaves under the same conditions suggest that the latter is better protected against oxidative damage under salt stress. Another explanation would be that unlike Korbous, Tabarka leaves escape from the applied stress. For instance, the two accessions could differ in their capacity to compartmentalize salt in their leaf cells, or to maintain the leaf water equilibrium. This point deserves further investigation.

The maintaining of leaf growth in Tabarka plants might be the result of the presence of more efficient antioxidative systems. The activities of antioxidant enzymes are determinant in the functioning of these systems. Under NaCl stress, the up-regulation of antioxidative enzymes characterized the response of the more tolerant ecotype. These enzymes include SOD and POD. SOD plays a significant role in protecting living cells against the toxicity of active O_2 species due to their capacity to scavenge superoxide (Scandalios, 1993). The effects of salinity stress on SOD activity has been reported in a number of plant species, lines and varieties. Thus, considerable variations in the activity of SOD in response to salinity appeared at the inter-specific or intra-specific level (Ashraf, 2009). In the present study, the activity of this enzyme increased in the leaves of the more tolerant ecotype (Tabarka), in the presence of NaCl, but no significant change in SOD activity was detected in the more sensitive ecotype (Korbous). Such a differential response to salt stress was reported between cultivars of potato (Rahnama and Ebrahimzadeh, 2005), rice (Dionisio-Sese and Tobita 1998), millet (Srenivasulu *et al.*, 2000), wheat (Sairam *et al.*, 2002), tomato (Shalata and Tal, 1998), strawberry (Turhan and Gulen Erics, 2008), cotton (Gossett *et al.*,1994) and beet (Bor *et al.*, 2003). In plants, scavenging of superoxides occurs by differential regulation of SOD isoforms (Wang et al., 2004). Studies on regulation of individual isoforms of SOD are therefore important because their role during salt stress could be elucidated (Jithesh *et al.*, 2006). However, all the previous reports reflect the effect of NaCl stress on total SOD activity. Most of the studies in halophytes have revealed increased activities of Mn SOD and Fe SOD isoforms localized in mitochondria and chloroplasts, respectively (Parida *et al.*, 2004; Wang *et al.*, 2004). This is not surprising because the immediate targets of salt stress are the chloroplasts and mitochondria. Superoxide radicals and other ROS are formed in the chloroplasts during photosynthesis, and could be translocated in mitochondria during oxygen reduction. However, superoxide radicals could also leak towards the cytosol, resulting in the induction of the cytosolic Cu-Zn SOD. Accordingly, our study revealed an increased activity of Cu-Zn SOD isoform in Tabarka ecotype in the presence de NaCl and suggested that superoxides were mainly over-produced in cytosol under salinity. Similar results were reported for the halophyte *Bruguiera gymnorrhiza* in presence of NaCl, mannitol and abscissic acid (Takemura *et al.*, 2000).

H_2O_2, which is toxic and produced by the activity of SOD to prevent cellular damage, must be eliminated by conversion to H_2O in subsequent reactions. CAT and APX regulate H_2O_2 level in plants (Mittler, 2002). Our data showed that CAT activities in leaves of both ecotypes decreased at 100 mM NaCl while APX activity did not change. Contrarily to our results, Shalata and Tal (1998), Bor *et al.* (2003), Vaidyanathan *et al.* (2003) and Sekmen *et al.* (2007) observed salt-induced activities of CAT and APX in salt-tolerant tomato, sugar beet, rice and plantain, respectively. It could be that, in our case, little H_2O_2 is formed in the leaves of salt-treated *C. maritimum*, so that CAT and APX activations are not required to detoxify H_2O_2. Another explanation could be the involvement of POD in H_2O_2 elimination since POD are known to participate in H_2O_2 scavenging. Salt tolerant plants often exhibit a stimulation of POD activities under salt stress (Bor *et al.*, 2003, Meloni *et al.*, 2003; Ben Amor *et al.*, 2006). Here, we obtained the same results with *Crithmum maritimum* plantlets. PODs are therefore probably the most important enzymes in the detoxification of H_2O_2 in that species. However, our results show that POD activity is constitutively higher in the more sensitive ecotype. Some studies reported that high levels of POD activity in condition of saline stress are correlated with a more reduced growth of plants and it appeared that POD

activity is involved in inhibiting the growth of aerial parts, rather than in protecting tissues against the accumulation of H_2O_2 (Lin and Kao, 2000). Such growth reduction has been attributed to the hemicellulose ferruloylation and the insolubilization of hydroxyproline rich glycoprotein (HPRG), those reactions being catalyzed by POD (Dionisio-Sese and Tobita, 1998). A similar role of POD has been proposed in cowpea plants subjected to NaCl (200 mM), where induction of POD activity in leaf tissues could be involved in stopping growth through the activation of tissue lignification (Cavalcanti *et al.*, 2004).
GR, the last enzyme of ascorbate–glutathione cycle, catalyzes NADPH-dependent reduction of oxidized glutathione. GR is important for plant protection against oxidative stress (Foyer and Halliwell, 1976). Although 100 mM NaCl treatment remarkably enhanced GR activity of Tabarka ecotype, slightly decreased that of Korbous ecotype. Because decreased GR activity enhances plant sensitivity to environmental stress (Aono *et al.*, 1995), the salt induced decrease in GR activity found in leaves of Korbous plants may explain the impairment of leaf growth and loss of membrane integrity. In the same way, oxidative stress in Tabarka appears to be prevented by the strong activity of SOD, POD and the ascorbate–glutathione cycle, as shown by the increased GR activities under 100 mM NaCl stress. The induction of GR by NaCl could increase the NADP / NADPH ratio, promoting the availability of NADP to accept electrons from the chain of electron transfer and limiting ROS levels in chloroplasts (Ben Amor *et al.*, 2006).

5. Conclusion

In the present study, plant growth and leaf antioxidant activities were analyzed in two Tunisian ecotypes of the halophyte *Crithmum maritimum* (Tabarka and Korbous, sampled from humid and semi-arid bioclimatic stages, respectively) under controlled salt stress.
Both ecotypes responded differently to low salt treatment. Korbous ecotype behaved like a facultative halophyte, with growth decreasing as NaCl levels rised in the medium. Conversely, biomass production in Tabarka ecotype was stimulated by 100 mM NaCl.
Our results showed also that intraspecific variability in *C. maritimum* salt tolerance coincides with differential antioxidant responses.
As a whole, our findings suggest the capacity of a plant ecotype or species to induce antioxidative enzymes in response to salt may greatly contribute to its ability to sustain growth.

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7. References

Aono, M.; Saji, H.; Fujiyama, K.; Sugita, M.; Kondo, N. & Tanaka, K. (1995). Decrease in activity of glutathione reductase enhances paraquat sensitivity in transgenic *Nicotiana tabacum*. *Plant Physiology* 107, 645–648.

Ashraf, M. (2009). Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotechnolgy Advances* 27, 84-93.

Ashraf, M. & Foolad, M.R. (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany* 59, 206-216.

Ashraf, M. & Harris, P.J.C. (2004). Potential biochemical indicators of salinity tolerance in plants. *Plant Science* 166, 3-16.

Beauchamp, C. & Fridovich, I. (1971). Superoxide dismutase: improved assays and applicable to acrylamide gels. *Analytical Biochemistry* 44, 276-287.

Ben Amor, N.; Ben Hamed, K.; Debez, A.; Grignon, C. & Abdelly, C. (2005). Physiological and antioxidant responses of the perennial halophyte *Crithmum maritimum* to salinity. *Plant Science* 168, 889–899.

Ben Amor, N.; Jiménez, A.; Megdiche, W.; Lundqvist, M.; Sevilla, F. & Abdelly, C. (2006). Response of antioxidant systems to NaCl stress in the halophyte *Cakile maritima*. *Physiologia Plantarum* 126, 446-457.

Ben Hamed, K.; Castagna, A.; Elkahoui, S.; Ranieri, A. & Abdelly, C. (2007). Sea fennel (*Crithmum maritimum* L.) under salinity conditions: a comparison of leaf and root antioxidant responses. *Plant Growth Regulation* 53, 185-194.

Bor, M.; Özdemir, F. & Turkan, I. (2003). The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet *Beta vulgaris* L. and wild beet *Beta maritima* L.. *Plant Science* 164, 77-84.

Bradford, M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72, 248-254

Cavalcanti, F.R.; Oliveira, J.T.A.; Martins-Miranda, A.S.; Viegas, R.A. & Silveira, J.A.G. (2004). Superoxide dismutase, catalase and peroxidases activities do not confer protection against oxidative damage in salt-stressed cowpea leaves. *New Phytologist* 163, 563-571.

Chaparzadeh, N.; D'Amico, M.L.; Khavari-Nejad, R.A.; Izzo, R. & Navarri-Izzo, F. (2004). Antioxidative response of *Calendula officinalis* under salinity conditions. *Plant Physiology and Biochemistry* 42, 695-701.

Cheeseman, J.M.; Herenedeen, L.B.; Cheeseman, A.T. & Clough, B.F. (1997). Photosynthesis and photoprotection in mangroves under field conditions. *Plant & Cell Environment* 20, 579-588.

Cherrian, S.; Reddy, M.P. & Pandya, J.B., (1999). Studies on salt tolerance of *Avicennia marina* (Forsk.) Vierh.: effect of NaCl on growth, ion accumulation and enzyme activity. *Indian Journal of Plant Physiology* 4, 266-270.

Dat, J.; Vandenabeele, S.; Vranova, E.; Van Montagu, M.; Inzé, A.D. & Van Breusegem, F. (2000). Dual action of the active oxygen species during plant stress responses. *Cell Molecular and Life Science* 57, 779-795.

Di Baccio, D.; Navari-Izzo, F. & Izzo, R. (2004). Seawater irrigation: antioxidant defense responses in leaves and roots of a sunflower (*Helianthus annuus L.*) ecotype. *Journal of Plant Physiology* 161, 1359-1366.

Dionisio-Sese, M.L. Tobita, S. (1998). Antioxidant responses of rice seedlings to salinity stress. *Plant Science* 135, 1–9.

Foyer, C.H. & Halliwell, B. (1976). The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. *Planta* 133, 21-25.

Foyer, C.H. & Noctor, G. (2000). Oxygen processing in photosynthesis: regulation and signalling. *New Phytologist* 146, 359-388.

Gossett, D.R.; Millhollon, E.P. & Lucas, M.C. (1994). Antioxidant response to NaCl stress in salt-tolerant and salt-sensitive cultivars of cotton. *Crop Science* 34, 706-714

Hagege, D.; Nouvelot, A. ; Boucaud, J. & Gaspar, T. (1990). Malondialdehyde titration with thiobarbiturate in plant extracts: avoidance of pigment interference. *Phytochemical Analysis* 1, 86-89.

Jithesh, M.N.; Prashanth, S.R.; Sivaprakash, K.R. & Parida, A.K. (2006). Antioxidative response mechanisms in halophytes: their role in stress defence. Journal of Genetics 85, 237-254.

Katsuhara, M.; Otsuka, T. & Ezaki, B. (2005). Salt stress-induced lipid peroxidation is reduced by glutathione S-transferase, but this reduction of lipid peroxides is not enough for a recovery of root growth in *Arabidopsis*. *Plant Science* 169, 369-373.

Ksouri, R.; Megdiche, W.; Debez, A.; Falleh, H.; Grignon, C. & Abdelly, C. (2007). Salinity effects on polyphenol content and antioxidant activities in leaves of the halophyte *Cakile maritima*. *Plant Physiology and Biochemistry* 45, 244-249.

Lin, C.C. & Kao, C.H. (2000). Effect of NaCl on H_2O_2 metabolism in rice leaves. *Plant Growth Regulation* 30, 151-155.

Lück, H. (1965). Catalase, In: *Methods of Enzymatic Analysis,* Bergmeyer, H.U. (Ed.), pp 885-888, Academic Press, New York.

Megdiche, W.; Ben Amor, N.; Debez, A.; Hessini, K.; Ksouri, R. & Abdelly, C. (2009). Physiological and biochemical traits involved in the genotypic variability to salt tolerance of Tunisian *Cakile maritima*. *African Journal of Ecology* 47, 774-783.

Meloni, D.A.; Oliva, M.A.; Martinez, C.A. & Cambraia, J. (2003). Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. *Environmental and Experimental Botany* 49, 69-76.

Mittler, R. (2002). Oxidative stress, antioxidant and stress tolerance. *Trends in Plant Science* 7, 405-410.

Nakano, Y. & Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplast. *Plant & Cell Physiology* 22, 867-880.

Navari-Izzo, F.; Quartacci, M.F.; Pinzino, C.; Dalla vecchia, F. & Sgherri, C.L.M. (1998). Thylakoid-bound and stromal antioxidative enzymes in wheat treated with excess copper. *Physiologia Plantarum* 104, 630-638.

Noctor, G. & Foyer, C.H. (1998). Ascorbate and glutathione: keeping active oxygen under control. *Annual Review of Plant Physiology and Plant Molecular Biology* 49, 623-647.

Parida, A.K. & Das, A.B. (2005). Salt tolerance and salinity effects on plants. *Ecotoxicology and Environmental Safety* 60, 324-349.

Parida, A.K.; Das, A.B. & Mohanty, P. (2004). Investigations on the antioxidative defence responses to NaCl stress in a mangrove, *Bruguiera parviflora*: differential regulations of isoforms of some antioxidative enzymes. *Plant Growth Regulation* 42, 213–226.

Price, A. and Hendry, G. (1991). Iron catalyzed oxygen radical formation and its possible contribution to drought damage in nine native grasses and three cereals. *Plant Cell Environment* 14, 477-481.

Rahnama, H. & Ebrahimzadeh, H. (2005). The effect of NaCl on antioxidant enzyme activity in potato seedlings. *Biologia Plantarum* 49, 93-99.

Ranieri, A. ; Petacco, F. ; Castagna, A. & Soldatini, G.F. (2000). Redox state and peroxidase system in sunflower plants exposed to ozone. *Plant Science* 159, 159-168.

Sairam, R.K.; Rao, K.V.; Srivastava, G.C. (2002). Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. *Plant Science* 163, 1037–1046.

Scandalios, J.G. (1993). Oxygen stress and superoxide dismutase. *Plant Physiology* 101, 7-12.

Scebba, F.; Sebastiani, L. & Vitagliano, C. (1999). Protective enzymes against activated oxygen species in wheat (*Triticum aestivum* L.) seedlings: Responses to cold acclimation. *Journal of Plant Physiology* 155, 762-768.

Sekmen, A.H.; Türkan, I. & Takio, S. (2007). Differential responses of antioxidative enzymes and lipid peroxidation to salt stress in salt-tolerant *Plantago maritima* and salt-sensitive *Plantago media*. *Physiologia Plantarum* 131, 399-411.

Shalata, A. & Tal, M. (1998). The effect of salt stress on lipid peroxidation and antioxidant in the leaf of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennellii*. *Physiologia Plantarum* 104, 169-174.

Shulavev, V. & Oliver, D.J. (2006). Metabolic and proteomic markers for oxidative stress. New tools for reactive oxygen species research. Plant Physiology 141, 367-372.

Sreenivasulu, N.; Grimm, B.; Wobus, U. & Weschke, W. (2000). Differential response of antioxidant compounds to salinity stress in salt tolerant and salt sensitive seedlings of foxtail millet (*Setaria italica*). *Physiologia Plantarum* 109, 435-442.

Taji, T.; Seki, M.; Sakuri, T.; Kobayashi, M.; Ishiyama, K.; Naruska, Y.; Zhu, J.K. & Shinosaki, K. (2004). Comparative genomics in salt tolerance between *Arabidopsis thaliana*-related halophyte salt cress using *Arabidopsis* microarray. *Plant Physiology* 135, 1697-1709.

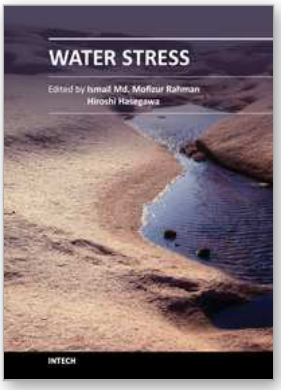
Takemura, T.; Hanagata, N.; Sugihara, K.; Baba, S.; Karube, I. & Dubinsky, Z. (2000). Physiological and biochemical responses to salt stress in the mangrove, *Bruguiera gymnorrhiza*. *Aquatic Botany* 68, 15-28.

Turhan, E. & Gulen Erics, A. (2008). The activity of antioxidative enzymes in three strawberry cultivars related to salt-stress tolerance. *Acta Physiologia Plantarum* 30, 201-208.

Vaidyanathan, H.; Sivakumar, P.; Chakrabarty, R. & Thomas, G. (2003). Scavenging of reactive oxygen species in NaCl-stressed rice (*Oryza sativa* L.) - differential response in salt-tolerant and sensitive varieties. *Plant Science* 165, 1411-1418.

Wang, B.; Luttge, U. & Ratajczak, R. (2004). Specific regulation of SOD isoforms by NaCl and osmotic stress in leaves of the C_3 halophyte *Suaeda salsa* L.. *Journal Plant Physiology* 161, 285-293.

Zarrouk, M.; El Almi, H.; Ben Youssef, N.; Sleimi, N.; Ben Miled, D.; Smaoui, A. & Abdelly, C. (2004). Lipid composition of seeds of local halophyte species: *Cakile maritima*, *Zygophyllum album* and *Crithmum maritimum*. In: Cash Crop Halophytes: Recent Studies, Lieth, H. (Ed.), Kluwer Academic Publishers, pp 121-126.



Water Stress

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Plants experience water stress either when the water supply to their roots becomes limiting, or when the transpiration rate becomes intense. Water stress is primarily caused by a water deficit, such as a drought or high soil salinity. Each year, water stress on arable plants in different parts of the world disrupts agriculture and food supply with the final consequence: famine. Hence, the ability to withstand such stress is of immense economic importance. Plants try to adapt to the stress conditions with an array of biochemical and physiological interventions. This multi-authored edited compilation puts forth an all-inclusive picture on the mechanism and adaptation aspects of water stress. The prime objective of the book is to deliver a thoughtful mixture of viewpoints which will be useful to workers in all areas of plant sciences. We trust that the material covered in this book will be valuable in building strategies to counter water stress in plants.

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