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Sea fennel (*Crithmum maritimum* L.) under salinity conditions: a comparison of leaf and root antioxidant responses

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Abstract The present study was carried out to compare the effect of NaCl on growth, cell membrane damage, and antioxidant defences in the halophyte *Crithmum maritimum* L. (sea fennel). Physiological and biochemical changes were investigated under control (0 mM NaCl) and saline conditions (100 and 300 mM NaCl). Biomass and growth of roots were more sensitive to NaCl than leaves. Roots were distinguished from leaves by increased electrolyte leakage and high malondialdehyde (MDA) concentration. Superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) activities, ascorbic acid (AA) and glutathione (GSH) concentrations were lower in the roots than in the leaves of control plants. The different activity patterns of antioxidant enzymes in response to 100 and 300 mM NaCl indicated that leaves and roots reacted differently to salt stress. Leaf CAT, APX and glutathione reductase (GR) activities were lowest at 300 mM NaCl, but they were unaffected by 100 mM NaCl. Only SOD activity was reduced in the latter treatment. Root SOD activity was significantly decreased in response to 300 mM NaCl and root APX activity was significantly higher in plants treated with 100 and 300 mM compared to the controls. The other activities in roots were insensitive to salt. The concentration of AA decreased in leaves at 100 and 300 mM NaCl, and in roots at 300 mM NaCl, when compared to control plants. The concentrations of GSH in NaCl-treated leaves and roots were not significantly different from the controls. In both organs, AA and GSH were predominating in the total pool in ascorbic acid and glutathione, under control or saline conditions.

Keywords Antioxidant enzymes · Ascorbic acid · *Crithmum maritimum* L. · Glutathione · Halophytes · Leaves · Oxidative stress · Roots · Salinity

Abbreviations

AA	Ascorbic acid
APX	Ascorbate peroxidase
CAT	Catalase
DHA	Dehydroascorbate
DHAR	Dehydroascorbate reductase
DW	Dry weight
EC	Electrical conductivity
EL	Electrolyte leakage
FW	Fresh weight
GR	Glutathione reductase
GSH	Reduced glutathione
GSSG	Oxidized glutathione
LP	Lipid peroxidation

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MDA	Malondialdehyde
MDHAR	Monodehydroascorbate reductase
RGR	Relative growth rate
ROS	Reactive oxygen species
SOD	Superoxide dismutase
v	Volume
w	Weight

Introduction

Under high salinity conditions, the growth and survival of plants depends on adaptations to both low water potentials and high sodium concentrations. High salinity in the external solution of plant cells imposes ion-specific stresses resulting from Na^+ and Cl^- concentrations and ion inbalance (Serrano et al. 1999). It also inflicts hyper osmotic shock on plants, as the chemical activity of water decreases causing the loss of cell turgor. Therefore, survival and growth depends on adaptation to re-establish homeostasis (Zhu 2001). Both a salt-induced reduction in chloroplast stromal volume and the generation of reactive oxygen species (ROS) can also play an important role in decreasing plant photosynthetic and respiratory capacities, thus inhibiting growth (Price and Hendry 1991). Plants are equipped with an array of non-enzymatic scavengers and antioxidant enzymes that act in concert to alleviate cellular damage under oxidative stress (Foyer and Noctor 2000). Superoxide dismutase (SOD) reacts with the superoxide radical to produce H_2O_2 (Scandalios 1993) which is scavenged by peroxidases, especially ascorbate peroxidase (APX) and catalase (CAT). CAT has been found predominantly in leaf peroxisomes where it basically functions to remove H_2O_2 formed in the photorespiration or in β-oxidation of fatty acids in the glyoxysomes (Dat et al. 2000). However, CAT activity is also found in the roots (Corpas et al. 1999; Shalata et al. 2001). APX which uses ascorbic acid (AA) as a reductant in the first step of the ascorbate glutathione cycle is the most important plant peroxidase in H_2O_2 detoxification (Foyer and Halliwell 1976). In addition, ROS are scavenged non-enzymatically by hydrophilic antioxidants, such as ascorbate (AA) and glutathione (GSH), found mainly in photosynthetic tissues, however these antioxidants have been detected in the roots of bean (Cuypers et al. 2000) and tomato (Shalata et al. 2001).

Increasing evidence indicates that changes in the concentrations of antioxidant molecules and enzyme activity are among the first signs of tolerance and/or adaptation to stress (Di Baccio et al. 2004). Various abiotic stresses, such as heavy metals (Rucinska et al. 1999), hypoxia (Biemelt et al. 2000), chilling (Queiroz et al. 1998) and salinity (Lee et al. 2001; Khan et al. 2002) have been shown to differently alter leaf and root antioxidant system activity. If NaCl, for example, is responsible for the modulation of these antioxidants, then different concentrations of NaCl stress should be expected to produce different degrees of effect.

Several studies have demonstrated that salt-tolerant species increase their antioxidant enzyme activities and antioxidant contents in response to salt stress, while salt-sensitive species failed to do so (Lopez et al. 1996; Meneguzzo et al. 1999; Shalata and Tal 1998). Recent studies show that even under control conditions, salt tolerant plants display a substantially higher abundance of major antioxidant components and expression of antioxidant enzyme activities than sensitive plants (Taji et al. 2004; M'rah et al. 2006).

In Tunisia, *Crithmum maritimum* L. (sea fennel) thrives in coastal bluff communities where the accumulation of salt spray on leaves is considerable (Orcut and Nilsen 2000). This species has the potential to grow in salt-affected areas (Shay 1990). Zarrouk et al. (2004) reported clearly that the quality of extracted oil from *C. maritimum* seeds is similar to that of Olive and Canola. This observation supports reports on the potential of this halophyte to act as a new source of edible oil (Weber et al. 2007). Leaves of *C. maritimum* contain a high concentration of ascorbic acid and traditionally have protected sailors from scurvy (Shay 1990).

Crithmum maritimum is a Na^+-accumulator halophyte (Ben Hamed et al. 2004; Ben Amor et al. 2005) and is reported to have efficient antioxidant enzyme systems which are crucial in the tolerance to salt accumulation in its leaves (Ben Amor et al. 2005, 2006). In the present study, we focus on the oxidative stress symptoms and compare enzymatic and non-enzymatic antioxidant activities in the roots and leaves of control and salt treated plants.

Materials and methods

Plant material and growth conditions

Seeds of *C. maritimum* were collected from the shores of Korbous, about 40 km in the south east of Tunis (bioclimatic semi arid stage). Seeds were sown in pots (two seeds per pot), filled with inert sand and irrigated with distilled water until germination. At the early development stages, seedlings were watered daily with Hewitt nutrient solution (pH 7.3, EC 2.7 mS cm^{-1}) (Hewitt 1966). About 2-month-old seedlings were divided into three blocks, which were treated with solutions of 0, 100, and 300 mM NaCl, respectively. Salt concentrations were increased daily by 50 mM NaCl, until reaching the required concentration. Plants were irrigated with their corresponding solutions every 2 days. Salt-treated plants were irrigated with distilled water between successive salt irrigations in order to eliminate excess in salt accumulated in the sand. Plant culture took place in a greenhouse at 25, 70% relative humidity (RH) during the day (16 h) and 15°C, 90% RH at night (8 h).

Growth activity

The relative growth rate (RGR) was calculated according to Hunt (1990).

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

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

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

H_2O_2 determination

The H_2O_2 concentration was measured colorimetrically (Chaparzadeh et al. 2004). H_2O_2 was extracted by homogenizing leaf and root materials with phosphate buffer (50 mM, pH 6.8) including 1 mM hydroxylamine. The homogenate was centrifuged at 6,000*g* for 25 min and 1 ml of the supernatant was added to 2 ml 0.1% titanium chloride in 20% (v/v) H_2SO_4. After centrifugation at 6,000*g* for 15 min, the absorbance of the supernatant was recorded at 410 nm. H_2O_2 concentration was calculated using the extinction coefficient 0.25 mM^{-1} cm^{-1}. H_2O_2 concentration was expressed on the basis of fresh weight (FW).

Lipid peroxidation

The extent of lipid peroxidation (LP) was estimated by determining the concentration of malondialdehyde (MDA) (Draper and Hadley 1990). Leaf and root material was homogenized in 0.1% (w/v) TCA solution. The homogenate was centrifuged at 15,000*g* for 10 min and 1 ml of the supernatant obtained was added to 4 ml 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. Samples were centrifuged at 10,000*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}.

Electrolyte leakage

The leaf and root samples (0.2 g) were placed in test tubes containing 10 ml of double distilled water. The leaves were cut into discs of uniform size (5 mm length). The tubes were incubated in a water bath at 32°C for 2 h and the initial electrical conductivity of the medium (EC1) measured. The samples were autoclaved at 121°C for 20 min to release all the electrolytes, cooled to 25°C and the final electrical conductivity (EC2) measured (Dionisio-Sese and Tobita 1998). The electrolyte leakage (EL) was calculated by using the formula: EL = (EC1/EC2) × 100.

Enzyme extractions

All of the following operations were performed at 4°C. The fresh leaf and root samples (0.5 g) were rapidly extracted in a pre-chilled mortar with 10% (w/w) 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 APX activity, 20 mM ascorbate was added to the extraction medium to maintain the enzyme active during extraction. The homogenate was centrifuged at 12,000*g* for 30 min. For APX, the supernatant was dialysed for 2 h against the same buffer used for the homogenization containing 5 mM sodium ascorbate. Three replicates per treatment were used. The supernatants were collected and their protein concentrations were determined according to Bradford (1976), using bovine serum albumin as a standard.

Enzyme assays

The total 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 reaction colour was measured spectrophotometrically at 560 nm. The control reaction mixture had no enzyme extract, while blanks had the same complete reaction mixture, but were kept in the dark. The volume of sample causing 50% inhibition of colour development was taken as one unit of SOD activity and the activity was expressed as units per mg of protein.

The total CAT (EC 1.11.1.6) activity was measured spectrophotometrically according to the method of Luck (1965), by monitoring the decline in absorbance at 240 nm as H_2O_2 was consumed, against a plant extract-free blank. The 3 ml reaction mixture contained 66 mM sodium phosphate buffer (pH 7.0), to which 30% (w/v) H_2O_2 was added (the optical density was around 0.5 at 240 nm 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. The time Δt required for a decrease in the optical density of from 0.45 to 0.4 was used for CAT activity calculations. CAT activity was expressed as unit per mg of protein. A unit is the amount of an enzyme which liberates half the peroxide oxygen from a hydrogen peroxide solution of any concentration in 100 s at 25°C.

The total 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 as ascorbate was oxidized ($\Sigma = 2.8\ mM^{-1}\ cm^{-1}$). The oxidation rate of ascorbate was estimated between 1 and 60 s after starting the reaction with the addition of H_2O_2. The 1 ml reaction mixture contained 50 mM HEPES-NaOH (pH 7.6), 0.22 mM ascorbate, 1 mM H_2O_2, and an enzyme sample. The control reaction mixture was prepared without the 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. The activity was expressed as units (µmol of oxidized ascorbate per min) per mg of protein.

The total GR (EC 1.6.4.2) activity was determined by following the rate of NADPH oxidation as measured by the decrease in the absorbance at 340 nm against a extract-free blank. The assay mixture (1.0 ml final volume) was composed of 0.4 mol/l potassium phosphate buffer, pH 7.5, 0.4 mmol l^{-1} Na_2EDTA, 5.0 mmol l^{-1} GSSG, 2.0 mmol l^{-1} NADPH, and 100 µl of crude extract. The reaction was initiated by NADPH addition and the GR activity was determined by monitoring GSSG-dependent oxidation of NADPH at 340 nm and 30°C (Di Baccio et al 2004). Corrections were made for the background absorbance at 340 nm, without NADPH. Activity was expressed as units (µmol of oxidized NADPH per min) per mg of protein.

Ascorbate determination

The aliquots of the fresh leaf and root tissues (0.5 g) were homogenized in ice-cold 6% (w/v) TCA, using a cold mortar and pestle. Total and reduced AA were determined in the supernatant after centrifugation at 15,000*g* for 10 min at 4°C. The assay is based on the reduction of Fe^{3+} to Fe^{2+} by AA in acid solution and the spectrophotometric detection of Fe^{2+} complexed with 2,2-dipyridyl (which absorbs at 525 nm). Total ascorbate was determined through a reduction of DHA to AA by 10 mM DTT. Excess DTT was removed with *N*-ethylmaleimide (NEM) 4% (w/v). A standard curve covering the range of 10–50 µmol AA was used (Kampfenkel et al. 1995).

Glutathione determination

The aliquots of the fresh leaf and root tissue (0.5 g) were homogenized in ice-cold 5% (w/v) TCA, using a cold mortar and pestle and centrifuged at 15,000*g* for 10 min at 4°C. Total (GSG + GSSG) and oxidized (GSSG) glutathione were determined in the supernatant by the 5,5-dithiobis-nitrobenzoic acid (DTNB)-GR recycling procedure as reported in Sgherri and Navari-Izzo (1995). Changes in the absorbance of the reaction mixture were measured at 412 nm at 25°C. The total glutathione concentration was calculated from a standard curve in which GSH equivalents (1–10 nmol) were plotted against the rate of change at 412 nm. GSSG was determined after GSH had been removed by 2-vinylpyridine derivatization. GSH was determined by subtracting GSSG, as GSH equivalents, from the total glutathione concentration.

Statistical analysis

Analysis of variance (ANOVA) using AV1W MSU-STAT 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) ($P \leq 0.05$).

Results

Biomass and growth of leaves and roots under NaCl conditions

Leaf biomass (FW and DW) and growth (RGR) were unaffected by 100 mM NaCl. However at 300 mM, they significantly decreased compared to the controls. Biomass and growth of roots were significantly reduced by 100 and 300 mM NaCl (Table 1). The decrease in RGR at 300 mM NaCl was more significant in roots than in leaves.

Oxidative stress in control and salt treated roots and leaves

H_2O_2 concentrations were higher in leaves than in roots (Table 2). They significantly increased with salt concentration. On the contrary, lipid peroxidation (LP) levels, as estimated from MDA concentrations, were significantly higher in roots than in leaves either under control or saline conditions (Table 2). The electrolyte leakage (EL) increased in leaves and roots with increasing salinity (Table 2).

Leaf and root antioxidant enzyme activities

Leaf CAT, APX and GR activities were significantly lower than the control in plants treated with 300 mM NaCl, but they were not affected by 100 mM NaCl. Only SOD activity was reduced in the latter treatment (Fig. 1). In roots, SOD and APX responded to NaCl. Root SOD activity was significantly decreased in response to 300 mM NaCl only, and root APX activity was significantly higher in plants treated with 100 and 300 mM compared to controls. The other activities in roots were insensitive to salt (Fig. 1).

Ascorbic acid (AA) and glutathione (GSH) concentrations under salinity conditions

Under control conditions, the concentration of AA was approximately 20-fold higher in leaves than in roots (Table 3). In leaves, the concentration of AA was significantly lower in plants treated with 100 and 300 mM NaCl than in control plants (Table 3). In roots, the concentration of AA in plants treated with 300 mM NaCl was 38 % lower than the control. The concentrations of GSH in salt-treated leaves and roots were not significantly different from the controls (Table 3). In both organs, AA and GSH were predominating in the total pool in ascorbic acid (AA + DHA) and glutathione (GSH + GSSG), either in control or saline conditions (Table 3).

Discussion

Differences in the leaf and root responses to NaCl

Moderate salinity (100 mM NaCl) reduced biomass or RGR of the roots only, whereas high salinity resulted in a significant reduction in both the leaves and the roots. This agrees with the assumption that roots, being the first part of the plant to encounter soil

Table 1 Biomass and RGR in control and NaCl treated leaves and roots

	Leaves			Roots		
	0 mM	100 mM	300 mM	0 mM	100 mM	300 mM
FW (g plant^{-1})	22.9 ± 5.1^{b}	26.4 ± 3.74^{b}	16.1 ± 4.1^{a}	5.4 ± 1.7^{c}	3.1 ± 0.9^{b}	1.0 ± 0.4^{a}
DW (g plant^{-1})	3.0 ± 0.5^{a}	3.0 ± 0.4^{a}	2.1 ± 0.5^{a}	0.7 ± 0.2^{b}	0.5 ± 0.1^{b}	0.2 ± 0.1^{a}
RGR (d^{-1})	0.034^{b}	0.034^{b}	0.027^{a}	0.047^{c}	0.040^{b}	0.022^{a}

Results are the means of five replicates of five plants each. Means followed by different letters are significantly different at $P \leq 0.05$ according to student's LSD test

Table 2 Measures of oxidative stress (hydrogen peroxide concentration, lipid peroxidation and electrolyte leakage) in control and NaCl treated leaves and roots

	Leaves			Roots		
	0 mM	100 mM	300 mM	0 mM	100 mM	300 mM
H_2O_2 (nmol g^{-1} FW)	64.5 ± 4.6^{c}	75.6 ± 3.5^{a}	82.6 ± 6.6^{a}	23.6 ± 1.4^{a}	25.2 ± 1.6^{a}	43.5 ± 1.0^{b}
MDA (nmol g^{-1} FW)	8.5 ± 1.7^{a}	7.5 ± 1.4^{a}	10.4 ± 1.3^{a}	17.3 ± 2.1^{b}	19.4 ± 2.0^{b}	24.2 ± 0.6^{c}
EL (%)	28 ± 3^{a}	32 ± 4^{ab}	46 ± 6^{c}	40 ± 7^{bc}	49 ± 6^{c}	62 ± 4^{d}

Results are the means of five replicates of five plants each. Means followed by different letters are significantly different at $P \leq 0.05$ according to student's LSD test

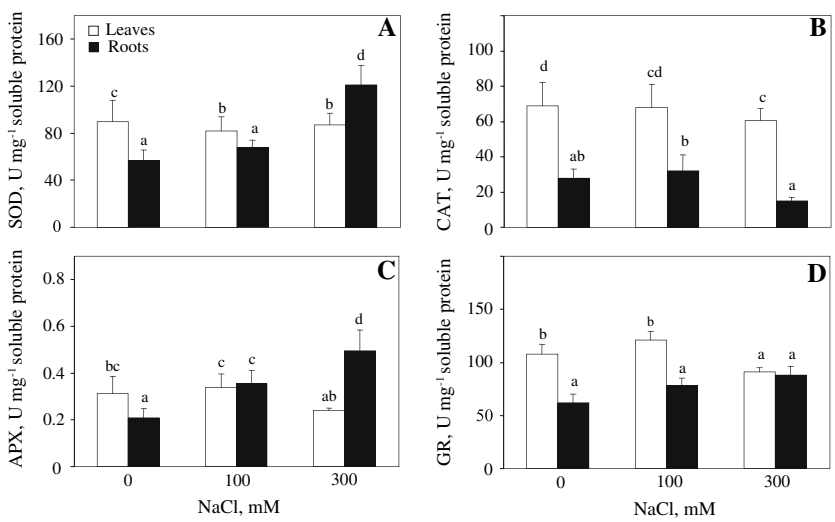

Fig. 1 Activities of SOD (**A**), CAT (**B**), APX (**C**) and GR (**D**) in the leaves and the roots of *C. maritimum* under control and saline conditions. Results are the means of five replicates of five plants each. Means followed by different letters are significantly different at $P \leq 0.05$ according to student's LSD test

Table 3 Ascorbic acid (AA) and glutathione (GSH) concentrations in control and NaCl treated leaves and roots

	Leaves			Roots		
	0 mM	100 mM	300 mM	0 mM	100 mM	300 mM
Reduced AA (nmol g^{-1} FW)	1488 ± 133^{d}	1045 ± 90^{bc}	669 ± 40^{a}	68 ± 7.8^{b}	64 ± 9.8^{b}	42 ± 2.9^{a}
AA + DHA (nmol g^{-1} FW)	1655 ± 167^{d}	1242 ± 135^{c}	851 ± 92^{ab}	73.4 ± 8.4^{b}	69 ± 10.7^{b}	49 ± 3.4^{a}
GSH (nmol g^{-1} FW)	129 ± 18^{abc}	126 ± 17^{ab}	109 ± 14^{a}	38 ± 7.8^{ab}	44 ± 9.8^{ab}	30 ± 2.9^{a}
GSH + GSSG (nmol g^{-1} FW)	171 ± 32^{c}	161 ± 33^{bc}	148 ± 30^{abc}	44 ± 9.2^{ab}	49 ± 11.4^{b}	37 ± 5.0^{ab}

Results are the means of three replicates of five plants each. Means followed by different letters are significantly different at $P \leq 0.05$ according to student's LSD test

salinity, are more markedly affected by saline conditions than are the leaves (Di Baccio et al. 2004; Ballesteros et al. 1997).

An increase in H_2O_2 and MDA concentration upon salt stress has been reported in different plant species (Dionisio-Sese and Tobita 1998; Lee et al. 2001, Sudhakar et al. 2001; Sairam and Srivastava 2002; Bandeoglu et al. 2004) and this has been shown to be related to stress levels and well correlated with lipid membrane damage. In our study, lipid membrane damage was only observed in the roots under high salinity condition, whereas leaves were less affected by the NaCl-induced oxidative stress than the roots. Mansour (1998) reported that the reduced membrane damage of onion leaf tissues under salinity stress could be explained by a reduction in the concentrations of H_2O_2 and MDA in leaf tissues. Lower lipid peroxidation under salt stress has also been reported in rice leaves (Dionisio-Sese and Tobita 1998), tomato (Shalata et al. 2001), sugar beet (Bor et al. 2003) and cotton (Meloni et al. 2003).

The origin of oxidative stress under salinity conditions is well documented in leaves where the inhibition of Calvin cycle results in over-reduction of oxygen and formation of superoxides (Grassmann et al. 2002). In roots, little is known about the origin of oxidative stress, although salt stress-related impairment of mitochondrial function is likely to be involved (Hamilton and Heckathorn 2001). Recent studies have revealed that salt-induced oxidative stress occurs in root mitochondria in a wild salt-tolerant tomato species as indicated by increased concentrations of H_2O_2 and MDA (Mittova et al. 2004). It is now widely accepted that superoxide radicals produced during respiration, in response to salt stress, are the main precursors of mitochondrial H_2O_2 and make an important contribution to the oxidative load experienced by the cell (Møller 2001). Root mitochondrial ROS production is increased as a result of high salinity (Gómez et al. 1999) as constraints are imposed on electron transport through mitochondrial complexes I and II (Hamilton and Heckathorn 2001).

Antioxidant responses of roots and leaves under control conditions

Recent studies have shown that even under control conditions salt tolerant plants display a substantially higher abundance of major antioxidant components along with an increased induction of antioxidant enzyme activity and gene expression compared to sensitive plants (Taji et al. 2004; M'rah et al. 2006). Taji et al. (2004) showed that the SOD gene induced by salt in *Arabidopsis* was overexpressed in unstressed conditions in *Thellungiella halophila*. In the same study, they also reported that *Thellungiella* was more tolerant not only to high salinity but also to oxidative stress. The specific SOD activity (120 U) measured in the control leaves of *C. maritimum* (Fig. 1) was 13 to 120-fold higher than the activities reported for wheat (Meneguzzo et al. 1999), pea (Hernandez et al. 1999) and cotton (Gossett et al. 1994), respectively. It was similar to SOD activity measured in salt tolerant species such as *Najas gramenia* (Rout and Shaw 2001), *Suaeda salsa* (Qiu Fang et al. 2005) and *Mesembryanthemum crystallinum* (Slesak et al. 2003). An elevated SOD activity could be considered as an advantage which allows the plants to resist potential oxidative damage without the requirement of further induction of SOD activity. These results are in agreement with those obtained by Stepien and Klobus (2005) where a positive correlation between salt tolerance of C4 plants, and high constitutive SOD activity was found. Like SOD, leaf CAT activity exceeded root activity under both control and stress conditions. CAT is crucial in removing photorespiratory H_2O_2 generated in C3 plants, under salinity conditions (Cavalcanti et al. 2004). Indeed, several experimental observations have conclusively demonstrated the necessity of sufficient CAT activity to cope with the abundant H_2O_2 production that accompanies the high photorespiratory flux (Foyer and Noctor 2003).

Gueta-Dahan et al. (1997) indicated that APX seems to be a key enzyme in determining the salt tolerance in citrus, as its constitutive activity was much higher in a salt tolerant cultivar. Our results demonstrated that the constitutive activity of APX was significantly greater in leaves than in the roots. The converse was reported in wheat (Meneguzzo et al. 1999) and lentil (Bandeoglu et al. 2004) where root tissues markedly exhibited higher APX activity compared to the shoot.

Antioxidant responses of roots and leaves at moderate and high salinity

Leaves and roots exhibited different responses to 100 mM NaCl with respect to the activities of SOD

and APX. SOD activity decreased significantly in leaves at 100 mM NaCl compared to the control, but not in roots. APX activity increased significantly in roots in response to 100 mM and was insensitive to this concentration in leaves. The activities of CAT and GR in the roots of plants treated with 100 mM NaCl were not different from those in control roots.

Both increase as well as decrease in the activity of SOD has been reported in plants in response to salinity stress (Dionisio-Sese and Tobita 1998; Shalata et al. 2001; Jitesh et al. 2006). It appears that the activity of SOD under salinity varies depending upon plant species, organ analyzed as well as the level of salinity (Chaparzadeh et al 2004). Decreased leaf SOD activity at 100 mM NaCl indicates that in leaves this enzyme is not crucial detoxification of ROS, and non enzymatic routes for conversion of $O_2^{\cdot-}$ to H_2O_2 via antioxidants such as GSH and AA may compensate for reduced activity of SOD. This view is supported from our results on GSH and AA concentrations under salt stress. At low and even high salinity, leaves exhibited higher concentrations of GSH and AA than roots. Increases in CAT and peroxidase activity is suggested to be an adaptive trait to overcome tissue damage of the metabolism by reducing toxic concentrations of H_2O_2 (Agarwal and Pandey 2004). In this study, the salt induced enhancement of APX (but not of CAT) in roots may suggest a more effective scavenging mechanism of APX than CAT in removing H_2O_2 and imparting tolerance against NaCl stress.

Several studies have implied that H_2O_2 can act as a signal of biotic and abiotic stress (Noctor and Foyer 1998). However, high concentrations of H_2O_2 directly damage organelles and accelerate Haber Weiss reactions, resulting in the formation of hydroxyl radicals ($OH^{\cdot}$) that can cause lipid peroxidation (Mittler 2002). Apart from causing peroxidation of membrane lipids, high concentrations of H_2O_2 cause a net reduction in photosynthesis (Vaidyanathan et al. 2003). Moreover, by channeling most of the photoreductants for the detoxification of H_2O_2 produced under stress and by shifting carbon photoassimilates to the production of defense and repair metabolites, plant growth under high salinity may undergo a significant reduction as shown here. In the roots of *C. maritimum*, The accumulation of H_2O_2 at 300 mM NaCl is suggested to be due to NaCl enhanced SOD and NaCl-deactivated CAT activities. Similarly, Elkahoui et al. (2005) reported that in salt sensitive cells of *Catharanthus roseus*, the strong induction in SOD activity was not accompanied by an increase in CAT activity. Clearly, the salt-induced stimulation of the APX activity was not sufficient to cope with the enhanced SOD activity and the reduced ability to scavenge H_2O_2 by CAT, probably leading to an increased generation of $OH^{\cdot}$. This is likely linked to the more drastic decrease in the root growth and the higher lipid peroxidation found in 300 mM NaCl-treated roots.

Conclusion

The present study shows an antioxidative response of *C. maritimum* to moderate and severe NaCl stress. The different activity patterns of antioxidant enzymes indicated that the leaves and the roots reacted differently to NaCl. The stimulated SOD and APX activities and the decreased CAT activities in the roots of the plants treated with 300 mM can be considered as response regulation under severe saline conditions. Besides, this study shows that the mechanisms which protect the plant against the salt-generated oxidative stress are more efficient in leaves than in roots. An important element of these mechanisms seems to be the higher constitutive antioxidant defense in leaves, as recently described for the model halophyte *Thellungiella halophila* (Taji et al. 2004; M'rah et al. 2006).

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References

Agarwal S, Pandey V (2004) Antioxidant enzyme responses to NaCl stress in *Cassia angustifolia*. Biol Plant 48:555–560

Ballesteros E, Blumwald E, Donnaire JP, Belver A (1997) Na^+/H^+ antiport activity in tonoplast vesicles isolated from sunflower roots induced by NaCl stress. Physiol Plant 99:328–334

Bandeoglu E, Eyidogan F, Yucel M, Oktem HA (2004) Antioxidant responses of shoots and roots of lentil to NaCl-salinity stress. Plant Growth Regul 42:69–77

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 Sci 168:889–899

Ben Amor N, Ben Hamed K, Ranieri A, Abdelly C (2006) Kinetics of the antioxidant response to salinity in *Crithmum maritimum*. In: Özturk M, Waisel Y, Khan MA, Görk G (eds) Biosaline agriculture and salinity tolerance in plants. Birkhauser Verlag, Switzerland, pp 81–86

Ben Hamed K, Debez A, Chibani F, Abdelly C (2004) Salt response of *Crithmum maritimum*, an oleaginous halophyte. Trop Ecol 45:151–159

Biemelt S, Keetman U, Mock HP, Grimm B (2000) Expression and activity of isoenzymes of superoxide dismutase in wheat roots in response to hypoxia and anoxia. Plant Cell Environ 23:135–144

Bor M, Ozdemir 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 Sci 164:77–84

Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Cavalcanti FR, Oliveira JTA, Martins-Miranda AS, Viégas RA, Silveira JAG (2004) Superoxide dismutase, catalase and peroxidase activities do not confer protection against oxidative damage in salt-stressed cowpea leaves. New Phytol 163:563–571

Chaparzadeh N, D'Amico ML, Khavari-Nejad RA, Izzo R, Navari-Izzo F (2004) Antioxidatve responses of *Calendula officinalis* under salinity conditions. Plant Physiol Biochem 42:695–701

Corpas FJ, Palma JM, Sandalio LM, Lopez-Huertas E, Romero-Puertas MC, Barroso JB, Del Rio LA (1999) Purification of catalase from pea leaf peroxisomes: identification of five different isoforms. Free Radical Res 31:S235–S241

Cuypers A, Vangronsveld J, Clijsters H (2000) Biphasic effect of copper on the ascorbate-glutathione pathway in primary leaves of *Phaseolus vulgaris* seedlings during the early stages of metal assimilation. Physiol Plant 110:512–517

Dat J, Vandenabeele S, Vranova E, Van Montagu M, Inzé D, Van Breusegem F (2000) Dual action of the active oxygen species during plant stress responses. Cell Mol Life Sci 57:779–795

Di Baccio D, Navari-Izzo F, Izzo R (2004) Seawater irrigation: antioxidant defence responses in leaves and roots of a sunflower (*Helianthus annuus* L.) ecotype. J Plant Physiol 161:1359–1366

Dionisio-Sese ML, Tobita S (1998) Antioxidant responses of rice seedlings to salinity stress. Plant Sci 135:1–9

Draper HH, Hadley M (1990) Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol 186:421–431

Elkahoui S, Hernandez JA, Abdelly C, Ghrir R, Limam F (2005) Effects of salt on lipid peroxidation and antioxidant enzyme activities of *Catharanthus roseus* suspension cells. Plant Sci 168:607–613

Foyer CH, Halliwell B (1976) Presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. Planta 133:21–25

Foyer CH, Noctor G (2000) Oxygen processing in photosynthesis: regulation and signalling. New Phytol 146: 359–388

Foyer CH, Noctor G (2003) Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. Physiol Plant 119:355–364

Gómez JM, Hernandez JA, Jimenez A, del Rio LA, Sevilla F (1999) Differential response of antioxidative system of chloroplasts and mitochondria to long-term NaCl stress of pea plants. Free Radical Res 31:11–18

Gossett DR, Millhollon EP, Lucas MC (1994) Antioxidant response to NaCl stress in salt-tolerant and salt-sensitive cultivars of cotton. Crop Sci 34:706–714

Grassmann J, Hippeli S, Elstner EF (2002) Plant's defence and its benefits for animals and medicine: role of phenolics and terpenoids in avoiding oxygen stress. Plant Physiol Biochem 40:471–478

Gueta-Dahan Y, Yaniv Z, Zilinskas BA, Ben-Hayyim G (1997) Salt and oxidative stress: similar and specific responses and their relation to salt tolerance in *Citrus*. Planta 203:460–469

Hamilton EW, Heckathorn SA (2001) Mitochondrial adaptations to NaCl. Complex I is protected by anti-oxidants and small heat shock Proteins, whereas complex II is protected by proline and betaine. Plant Physiol 126:1266–1274

Hernandez JA, Campillo A, Jimenez A, Alarcon JJ, Sevilla F (1999) Responses of antioxidant systems and leaf water relations to NaCl stress in pea plants. New Phytol 141:241–251

Hewitt EJ (1966) Sand and water culture methods used in the study of plant nutrition. Commw Bur Hortic Tech Commun 22:431–446

Hunt R (1990) Basic growth analysis. Plant growth analysis for beginners. Unwin Hyman, London

Jitesh MN, Prashanth SR, Sivaprakash KR, Parida AK (2006) Antioxdative response mechanisms in halophytes: their role in stress defence. J Genet 85:237–254

Kampfenkel K, Van Montagu M, Inze D (1995) Extraction and determination of ascorbate and dehydrascorbate from plant tissue. Anal Biochem 225:165–167

Khan MH, Singha KLB, Panda SK (2002) Changes in antioxidant levels in *Oryza sativa* L. roots subjected to NaCl salinity stress. Acta Physiol Plant 24:145–148

Lee DH, Kim YS, Lee CB (2001) The inductive responses of antioxidant enzymes by salt stress in rice (*Oryza sativa* L.). J Plant Physiol 158:737–745

Lopez F, Vansuyt G, Casse-Delbart F, Fourcroy P (1996) Ascorbate peroxidase activity, not the mRNA level, is enhanced in salt-stressed *Raphanus sativus* plants. Physiol Plant 97:13–20

Luck H (1965) Catalase. In: Bergmeyer HU (ed) Methods of enzymatic analysis. Verlag Chemie Academic Press, New York, pp 885–888

M'rah S, Ouerghi Z, Eymery F, Rey P, Hajji M, Grignon C, Lachaal M (2006) Efficiency of biochemical protection against toxic effects of accumulated salt differentiates

Thellungiella halophila from *Arabidopsis thaliana*. J Plant Physiol 164:375–384

Mansour MMF (1998) Protection of plasma membrane of onion epidermal cells by glycinebetaine and proline against NaCl stress. Plant Physiol Biochem 155:767–772

Meloni DA, Oliva MA, Martinez CA, Cambraia J (2003) Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. Environ Exp Bot 49:69–76

Meneguzzo S, Navario-Izzo F, Izzo R (1999) Antioxidative responses of leaves and roots of wheat to increasing NaCl concentrations. J Plant Physiol 155:274–280

Mittler R (2002) Oxidative stress, antioxidant and stress tolerance. Trends Plant Sci 7:405–410

Mittova V, Guy M, Tal M, Volokita M (2004) Salinity upregulates the antioxidative system in root mitochondria and peroxisomes of the wild salt-tolerant tomato species *Lycopersicon pennellii*. J Exp Bot 55:1105–1113

Møller IM (2001) Plant mitochondria and oxidative stress: electron transport, NADPH turnover, and metabolism of reactive oxygen species. Ann Rev Plant Physiol Plant Mol Biol 52:561–591

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

Noctor G, Foyer CH (1998) Ascorbate and glutathione: keeping active oxygen under control. Ann Rev Plant Physiol Plant Mol Biol 49:249–279

Orcutt DM, Nilsen ET (2000) Physiology of plants under stress—soil and biotic factors. John Wiley Sons, New York

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

Qiu-Fang Z, Yuan-Yuan L, Cai-Hong P, Cong-Ming L, Bao-Shan W (2005) NaCl enhances thylakoid-bound SOD activity in the leaves of C3 halophyte *Suaeda salsa* L. Plant Sci 168:423–430

Queiroz CGS, Alonso A, Mares-Guia M, Magalhaes AC (1998) Chilling-induced changes in membrane fluidity and antioxidant enzyme activities in *Coffea arabica* L. roots. Biol Plant 41:403–413

Rout NP, Shaw BP (2001) Salt tolerance in aquatic macrophytes: possible involvement of the antioxidative enzymes. Plant Sci 160:415–423

Rucinska R, Waplak S, Gwozdz E (1999) Free radical formation and activity of antioxidant enzymes in lupin roots exposed to lead. Plant Physiol Biochem 37:187–194

Sairam RK, Srivastava GC (2002) Changes in antioxidant activity in sub-cellular fractions of tolerant and susceptible wheat genotypes in response to long term salt stress. Plant Sci 162:897–904

Scandalios JG (1993) Oxygen stress and superoxide dismutase. Plant Physiol 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. J Plant Physiol 155:762–768

Serrano R, Mulet JMM, Rios G, Marquez JA, de Larrinoa IF, Leube MP, Mendizabal I, Pascual-Ahuir A, Proft M, Ros R, Montesinos C (1999) A glimpse of the mechanisms of ion homeostasis during salt stress. J Exp Bot 50: 1023–1036

Sgherri CLM, Navari-Izzo F (1995) Sunflower seedlings subjected to increasing water deficit stress: oxidative stress and defence mechanisms. Physiol Plant 93:25–30

Shalata A, Mittova V, Volokita M, Guy M, Tal M (2001) Responses of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon esculentum* to salt oxidative stress: the root antioxidative system. Physiol Plant 112:487–494

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*. Physiol Plant 104:169–174

Shay EG (1990) Saline agriculture. Salt tolerant plant for developing countries. Report of a panel of the Board on Science and Technology for International Development Office of International Affairs Research. National Academy Press

Slesak I, Miszalski Z (2003) Superoxide dismutase-like protein from roots of the intermediates C3-CAM plant *Mesembryanthemum crystallinum* L. in in vitro culture. Plant Sci 164:497–505

Stepien P, Klobus G (2005) Antioxidant defense in the leaves of C_3 and C_4 plants under salinity stress. Physiol Plant 125:31–40

Sudhakar C, Lakshmi A, Giridarakumar S (2001) Changes in the antioxidant enzymes efficacy in two high yielding genotypes of mulberry (*Morus alba* L.) under NaCl salinity. Plant Sci 161:613–619

Taji T, Seki M, Satou M, Sakurai T, Kobayashi M, Ishiyama K, Narusaka Y, Narusaka M, Zhu J-K, Shinozaki K (2004) Comparative genomics in salt tolerance between arabidopsis and arabidopsis-related halophyte salt cress using arabidopsis microarray. Plant Physiol 135:1697–1709

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 Sci 165: 1411–1418

Weber DJ, Ansari R, Gul B, Khan MA (2007) Potential of halophytes as source of edible oil. J Arid Environ 68: 315–321

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: Lieth H (ed) Cash crop halophytes: recent studies. Kluwer Academic Publishers Group, Dordrecht, pp 121–126

Zhu JK (2001) Plant salt tolerance. Trends Plant Sci 2:66–71