Acta Physiol Plant (2012) 34:1873–1881
DOI 10.1007/s11738-012-0985-z

ORIGINAL PAPER

Changes in the antioxidative systems of *Ocimum basilicum* L. (cv. Fine) under different sodium salts

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Received: 3 October 2011 / Revised: 14 March 2012 / Accepted: 16 March 2012 / Published online: 4 May 2012

Abstract The effects of different sodium salts on some physiological parameters and antioxidant responses were investigated in a medicinal and aromatic plant, *Ocimum basilicum* L. (cultivar Fine). Plants were subjected to an equimolar concentration of Na_2SO_4 (25 mM) and NaCl (50 mM) for 15 and 30 days. Growth, oxidative stress parameters [electrolyte leakage, peroxidation, and hydrogen peroxide (H_2O_2) concentration], antioxidant enzyme activities [ascorbate peroxidase (APX, EC 1.11.1.11), glutathione reductase (GR, EC 1.6.4.2), and peroxidases (POD, EC 1.11.1.7)], as well as antioxidant molecules [ascorbate and glutathione] were determined. The two salts affected leaf growth rates to the same extent, after 15 or 30 days of treatment, indicating a similar effect of Na_2SO_4 and NaCl salinity on growth, even if different (enzymatic and non-enzymatic) antioxidant mechanisms were involved in H_2O_2 detoxification. However, under both salts, the efficiency of the antioxidant metabolism seemed to be sufficient to avoid the deleterious effects of reactive oxygen species (ROS). Indeed, both ion leakage and peroxidation did not change under either Na_2SO_4 or NaCl salinity. As a whole, these data suggest that a cooperative process between the antioxidant systems is important for the tolerance of *Ocimum basilicum* L., cv. Fine to Na_2SO_4 and NaCl salinity.

Keywords Antioxidants · Growth · *Ocimum basilicum* L. · Reactive oxygen species · Salinity

Abbreviations

APX	Ascorbate peroxidase
AsA	Ascorbate
DHA	Dehydroascorbate
DTNB	5,5′-Dithiobis-nitrobenzoic acid
DW	Dry weight
Na_2EDTA	Disodium ethylenediamine-tetraacetic acid
FW	Fresh weight
GR	Glutathione reductase
GSH	Reduced glutathione
GSSG	Oxidized glutathione
H_2O_2	Hydrogen peroxide
LA	Leaf area
POD	Peroxidase
RLR	Relative ion leakage ratio
ROS	Reactive oxygen species
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reacting substances
TCA	Trichloroacetic acid

Introduction

Crop growth and productivity are adversely affected by salinity, which is one of the most serious problems for agriculture. It is expected to result in the loss of up to 50 % fertile land by the middle of the twenty-first century (Manchanda and Garg 2008). The loss in plant productivity

Communicated by G. Bartosz.

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due to salinity is the consequence of three main mechanisms: ion toxicity, nutritional disruption, and osmotic stress (Munns 2002), resulting in the overproduction of reactive oxygen species (ROS). Although ROS production is a phenomenon of aerobic metabolic process in chloroplast, mitochondria, and peroxisomes under normal physiological conditions, their overproduction causes oxidative damage and affects cell membrane properties (Ahmad et al. 2008).

To avoid the damage caused by ROS, plants have evolved enzymatic and non-enzymatic systems to scavenge active oxygen species (Alscher et al. 2002). Glutathione (GSH), ascorbate (AsA), carotenoids, and tocopherols are non-enzymatic antioxidants (Gupta et al. 2005), whereas enzymatic defenses include peroxidases (POD), superoxide dismutase (SOD), and catalase (CAT), which together with the other enzymes of the ascorbate–glutathione cycle such as ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) promote the scavenging of ROS (Cavalcanti et al. 2004).

PODs are widely distributed in all higher plants and protect cells against the destructive influence of H_2O_2 by catalyzing its decomposition (Lin and Kao 2002). APX is the most important peroxidase in H_2O_2 detoxification, catalyzing its reduction to water using the reducing power of ascorbate (Noctor and Foyer 1998). GR can also remove H_2O_2 via the ascorbate–glutathione cycle to maintain a high level of reduced ascorbate within chloroplasts.

Ascorbate is not only an efficient chemical scavenger of toxic free radicals, but also a required molecule for the formation of zeaxanthin, the most important quencher of excess light energy (Demmig-Adams and Adams 1994), and for the regeneration of α-tocopherol, an important protectant of thylakoid membranes (Fryer 1992). During these processes, ascorbate is oxidized to dehydroascorbic acid and can be regenerated by glutathione.

Glutathione is a water-soluble tripeptide containing a sulfhydryl group and has several important roles (Asada 2006). Reduced glutathione is the substrate for dehydroascorbate reductase in the ascorbate–glutathione cycle. The oxidized product (GSSG) is re-reduced by the flavoprotein GR using NADPH as an electron donor (Foyer 1997). Glutathione is found in chloroplasts at the concentration of 1–5 mM and, under physiological conditions, the ratio of the reduced (GSH) to oxidized (GSSG) glutathione is kept high, around 10:1 (Asada 1999). GSH scavenges hydroxyl radicals and singlet oxygen and may protect enzyme thiol groups (Hideg 1999). Maintaining and regeneration of a high concentration of reduced AsA and GSH, as well as upregulation of the activity of enzymes involved in their regeneration, could help in reducing the harmful effects of ROS generated during salt stress. The correlation between antioxidant capacity and salt tolerance is well known. Plants with high levels of constitutive or induced antioxidants have been reported to have higher resistance to oxidative damage (D'Amico et al. 2003; Chaparzadeh et al. 2004; Pérez-López et al. 2009, 2010).

Basil (*Ocimum basilicum* L.) possesses potential medicinal, pharmaceutical, and food industrial interests. It is cultivated in Mediterranean countries and in various regions, where salinity stress is common. In most cases, salinity problems are linked to an excess of NaCl in the irrigation water, but sometimes other salts, like Na_2SO_4, are present. In spite of its economic importance, relatively little work has been done on the physiological responses of basil to NaCl salinity and information in relation to Na_2SO_4 salinity is scanty. The majority of the studies carried out on this plant exclusively deal with its capacity to produce flavors and essential oils. Recently, a study conducted on *O. basilicum* comparing two cultivars differing in leaf size named Genovese (large size leaf) and Fine (small size leaf) showed that there was an intraspecific variability for biomass production accompanied by a modest but real variability in tolerance to NaCl aggression (Attia et al. 2010). Because of the fact that only few data are available on the physiological and antioxidative responses of basil to NaCl or Na_2SO_4 salinities, we explored in a previous paper (Tarchoune et al. 2010) the response of Genovese to both salts to gain information about the antioxidative response of this cultivar that was more evident under NaCl than under Na_2SO_4 stress.

The aim of the present work was, therefore, to investigate in detail how Fine cultivar responds to sodium chloride and sodium sulfate salinities. We were interested in finding out whether growth and antioxidant system responses are differently affected by the two salts. Therefore, the main objective of the present study was to analyze if there were differences in the antioxidative response of Fine cultivar to salinity stress caused by Na_2SO_4 and NaCl.

Materials and methods

Plant growth

Ocimum basilicum L. (Fine cultivar) seeds were germinated in the dark for 5 days in a polyester shelf containing rockwool as inert substrate with distilled water in a growth chamber at 25/20 °C (day/night). Seven-day-old seedlings obtained from seed germination were grown in diluted Hoagland's nutrient solution during the first week of growth. Then, selected uniform seedlings were transferred onto floating polyester in plastic pots, at a density of 24 plants per pot, filled with 65 L of aerated Hoagland's

solution diluted eightfold and renewed every 2 weeks. Salinization was initiated 4 weeks after sowing, lasting 15 and 30 days, by adding equimolar concentrations of different salts (25 mM Na_2SO_4 or 50 mM NaCl). Plant cultivation was carried out in May–July 2007 under greenhouse conditions. Day/night temperature was 29/18 °C, relative humidity was 70–90 %, global daily radiation was 10 MJ/m^2 and photon flux density was 500–700 μmol m^{-2} s^{-1}.

Growth analysis

At each sampling date, six different samples from the control and treatments were taken and each sample was analyzed in duplicate. Leaf dry weight (DW) (dried at 70 °C for 48 h) and leaf number of each plant were determined. Leaf area was measured using the Optimas software (Optimas Corporation, OPTIMAS^{TM}, version 6.1) after having scanned the leaves of each plant.

Thiobarbituric acid reactive substance assays

The thiobarbituric acid reactive substances (TBARS) were determined and calculated according to Hodges et al. (1999). Leaf samples were homogenized in ethanol:water (80:20; v:v) with inert sand using a mortar and pestle. The homogenate was centrifuged at 3,000*g* for 15 min.

A 200-ml supernatant aliquot was added to 1-ml solution containing 20 % (w/v) trichloroacetic acid and 0.01 % butylated hydroxytoluene with (+TBA solution) or without (–TBA solution) 0.5 % TBA. Each mixture was heated at 95 °C in a water bath for 30 min and then quickly cooled in an ice bath. After centrifugation at 12,000*g* for 15 min, absorbance of the supernatant was read at 535, 600, and 440 nm.

Electrolyte leakage

Samples of 25 leaf discs were immersed in 25 ml of double-distilled water and shaken for 1 h at room temperature. Electrical conductivity of the solution ($C_{initial}$) was measured using a conductometer (Metrohm 660 conductometer). Leaf discs were then killed in liquid nitrogen for 10 min. The conductivity of killed tissues (C_{max}) was measured after an additional hour of shaking. Relative ion leakage ratio (RLR) was calculated as the percentage of $C_{initial}$ over C_{max} (Quartacci et al. 2002).

H_2O_2 determination

H_2O_2 contents were evaluated following the method of Sgherri and Navari-Izzo (1995), using 1 g of tissue, a standard curve in the 1–15 nmol ml^{-1} H_2O_2 range at 0–4 °C and ice-cold solutions to immediately stop cellular metabolism. According to these authors, this method is very sensitive, reproducible and excludes the interference of other peroxides, except a small amount of lipid peroxide.

Enzyme extraction

All of the following operations were performed at 4 °C. Leaf samples were ground in a mortar with liquid nitrogen and extracted in 50 mM potassium phosphate (pH 7) containing 1 mM AsA for APX and POD. The extraction of GR was performed in 0.1 M potassium phosphate (pH 7.5) containing 0.5 mM Na_2EDTA. The homogenates were centrifuged at 12,100*g* for 15 min at 4 °C and the supernatants were collected and used for enzyme assays.

Enzyme assays

APX activity was measured by following the oxidation of AsA, operated by H_2O_2, at 290 nm and 25 °C according to Wang et al. (1991). Constant rate was calculated using the extinction coefficient of 0.0028 μM^{-1} cm^{-1} and corrected for the oxidation of ascorbate in the absence of H_2O_2. One APX unit was defined as the amount of enzyme required to oxidize 1 nmol (ascorbate) min^{-1}.

POD activity was determined at 25 °C following the increase in A_{430}. Each sample (1 ml) of reaction mixture contained 10 mM pyrogallol, 1.7 mM H_2O_2 and leaf extract in 0.1 M potassium phosphate (pH 6.5). Calculations were performed using an extinction coefficient of 2.47 mM^{-1} cm^{-1}. One POD unit was defined as the amount of enzyme required to decompose 1 μmol (H_2O_2) min^{-1}.

GR activity was assayed by the DTNB method that was performed by following the increase in the absorbance at 412 nm and 30 °C according to Smith et al. (1988). One GR unit was defined as the amount of enzyme required to oxidize 1 nmol (NADPH) min^{-1}.

Ascorbate determination

Fresh leaf tissue was immediately homogenized at 4 °C in 6 % (w/v) trichloroacetic acid. After centrifugation at 12,100*g* for 15 min, total (AsA + DHA) and reduced (AsA) ascorbate were determined in the supernatants according to the method of Kampfenkel et al. (1995). A standard curve covering the range of 5–50 nmol AsA was used.

Glutathione determination

Fresh leaf tissue was immediately homogenized at 4 °C in 6 % (w/v) TCA using a cold mortar and pestle. GSSG was

determined after removal of GSH from the extract by 2-vinylpyridine derivatizations according to Anderson (1985). Total glutathione (GSH + GSSG) and GSSG were determined in the supernatant by the 5,5′- dithio-bis-nitrobenzoic acid (DTNB)-GSSG reductase recycling procedure as reported by Anderson et al. (1992) and their contents were calculated as previously reported by Sgherri et al. (1994).

Statistical analysis

At each sampling date, six or three different samples used for physiological and biochemical analysis, respectively, were taken and each sample was analyzed in duplicate. All data are reported as mean value ± SE. One-way analysis of variance (ANOVA) was independently applied to the data to evaluate salt and treatment period effects. Statistical assessments of differences between mean values were performed by Duncan's multiple range test at $P \leq 0.05$. When necessary, an arc sin or angular transformation was applied before statistical analysis.

Results

Plant growth

After 15 days of treatment, dry biomass (Fig. 1a) and leaf area (Fig. 1b) and number (Fig. 1c) did not show any change in plants treated with 25 mM Na_2SO_4 or 50 mM NaCl. After 30 days of treatment, dry biomass and leaf area decreased at a similar extent under both salts in comparison with the control (about −44 and −25 %, respectively), whereas leaf number did not change under both salts.

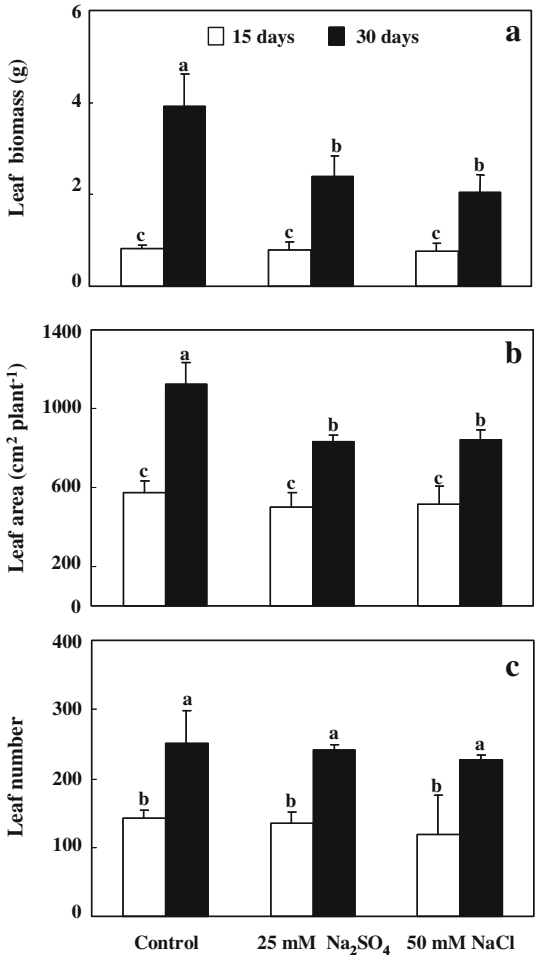

Fig. 1 Effects of 25 mM Na_2SO_4 and 50 mM NaCl on leaf biomass (**a**), area (**b**) and number (**c**) in basil (*Ocimum basilicum* L.; cultivar Fine) after 15 and 30 days of treatment. Data are means of six replicates ± SE that were analyzed in duplicate. For each parameter, different letters indicate significant differences at $P \leq 0.05$ as determined by Duncan's multiple range test

Oxidative stress evaluation

RLR and TBARS (Table 1) remained constant independently of treatment and harvest date. In contrast, H_2O_2 content (Table 1) showed significant increases in plants treated with NaCl (+34 and +64 %, after 15 and 30 days of treatment, respectively). However, in plants treated with Na_2SO_4, H_2O_2 content remained unchanged in comparison with the control after 15 or 30 days of treatment.

Antioxidative enzymes

APX activity (Fig. 2a) remained constant after 15 days of treatment in plants treated with Na_2SO_4 and increased two times in those treated with NaCl. After 30 days, APX activity increased in plants treated with 25 mM Na_2SO_4 compared to the control and decreased in those treated with 50 mM NaCl. GR activity (Fig. 2b) was improved after 15 days of treatment in plants subjected to both Na_2SO_4 (+182 %) and NaCl (+237 %) salts, whereas it remained unaltered after 1 month of treatment. POD activity (Fig. 2c) decreased at the first harvest (15 days of treatment) regardless of the salt type. At the second harvest, however, it increased by 33 % in plants treated with Na_2SO_4 and decreased by 36 % in those treated with NaCl.

Ascorbate and glutathione

After 15 days of treatment, both salts decreased total ascorbate (AsA + DHA) and AsA contents, as well as AsA/DHA ratio but increased DHA contents (Table 2). In contrast, after 30 days, neither NaCl nor Na_2SO_4 showed significant effect on total ascorbate (AsA + DHA). AsA content and AsA/DHA ratio remained constant in

Table 1 Effect of 25 mM Na_2SO_4 or 50 mM NaCl on relative ion leakage ratio (RLR), thiobarbituric acid reactive substances (TBARS) and hydrogen peroxide (H_2O_2) content in leaves of basil (*Ocimum basilicum* L.), cultivar Fine, after 15 and 30 days of treatment

	15 days			30 days		
	0 mM	25 mM Na_2SO_4	50 mM NaCl	0 mM	25 mM Na_2SO_4	50 mM NaCl
RLR (100 %)	4.6 ± 1.14[a]	3.9 ± 0.81[a]	3.8 ± 0.28[a]	4.5 ± 1.10[a]	4.4 ± 0.77[a]	4.4 ± 0.87[a]
TBARS (μmol g^{-1} DW)	138.9 ± 14.9[a]	129.6 ± 21.1[a]	118.5 ± 22.6[a]	132.3 ± 17.9[a]	146.3 ± 8.62[a]	149.1 ± 27.4[a]
H_2O_2 (μmol g^{-1} DW)	85.0 ± 9.56[c]	93.8 ± 2.74[c]	113.6 ± 5.45[b]	110.0 ± 11.5[b]	128.1 ± 31.6[b]	179.7 ± 11.8[a]

Each value is the mean of three replicates that were each analyzed in duplicate. For each parameter, the different letters indicate significant differences at $P \leq 0.05$, between both salt and period of treatment, as determined by Duncan's multiple range test

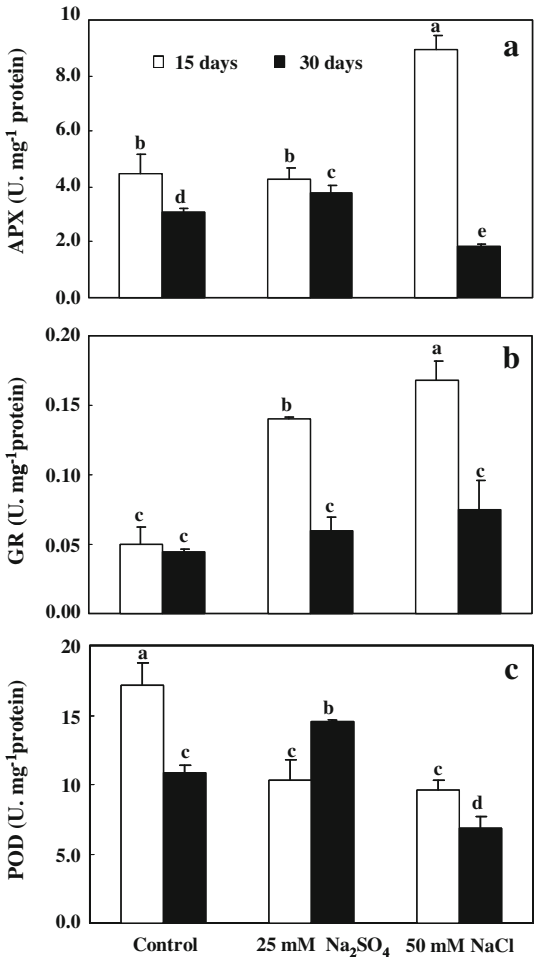

Fig. 2 Effect of 25 mM Na_2SO_4 or 50 mM NaCl on the activities of APX (**a**), GR (**b**) and POD (**c**) in leaves of basil (*Ocimum basilicum* L.; cultivar Fine) after 15 and 30 days of treatment. Data are means of three replicates ± SE that were analyzed in duplicate. For each parameter, different letters indicate significant differences at $P \leq 0.05$ as determined by Duncan's multiple range test

comparison with the control in plants treated with NaCl, whereas an increase in its content was observed in those treated with Na_2SO_4. Moreover, DHA content (Table 2) decreased in comparison with the control under both treatments. Total glutathione (GSH + GSSG) content (Table 3) showed an increase after 15 days in plants treated with NaCl, whereas in those treated with 25 mM Na_2SO_4, no significant variation was observed. As for GSH level, its increase was considerable in salt-treated plants, in particular those grown at 50 mM NaCl (+237 %). Regarding GSSG, a decrease in its content was observed under both treatments. As a consequence, GSH/GSSG ratio increased five times in salt-treated plants. After 30 days of treatment, no effect was observed on total glutathione (GSH + GSSG), GSH and GSSG contents and on GSH/GSSG ratio (Table 3) in plants subjected to salt treatments.

Discussion

Short-term effects of mild salt stress on growth and antioxidant systems

Salt tolerance has usually been assessed as the percentage of biomass production in saline versus control conditions over a prolonged period of time (Munns 2002). As indicated by leaf dry weight, leaf area and leaf number (Fig. 1a–c), no difference in growth was observed after 15 days of treatment between the control and salt-treated plants. The fact that Fine cultivar maintained substantially unchanged growth activity under Na_2SO_4 and NaCl salinities suggests its relative tolerance to both salts as compared to Genovese cultivar that showed more depressed growth by 25 mM Na_2SO_4 than by 50 mM NaCl (Tarchoune et al. 2010).

Several studies have shown that environmental stresses stimulate ROS production (Sgherri and Navari-Izzo 1995; Sgherri et al. 2007), and may induce oxidative damage to lipids, proteins and nucleic acids, and affect cell membrane properties (Ahmad et al. 2008). The fact that RLR and TBARS did not change in *O. basilicum* cultivar Fine (Table 1), following the treatments, may be an evidence of tolerance to salinity due to the adequate response of the antioxidative systems. This is in agreement with the findings of Chaparzadeh et al. (2004) and Pérez-López et al. (2009), who found that stability of biological membranes is a proof of salinity stress resistance.

Table 2 Effect of 25 mM Na_2SO_4 or 50 mM NaCl on total ascorbate (AsA + DHA), reduced (AsA) ascorbate, oxidized ascorbate (DHA) contents and reduced ascorbate (AsA)/dehydroascorbate (DHA) ratio in leaves of basil (*Ocimum basilicum* L.), cultivar Fine, after 15 and 30 days of treatment

	15 days			30 days		
	0 m M	25 mM Na_2SO_4	50 mM NaCl	0 mM	25 mM Na_2SO_4	50 mM NaCl
AsA + DHA	46.6 ± 1.00^{ab}	33.4 ± 0.39^{c}	44.4 ± 0.32^{b}	56.4 ± 6.30^{a}	55.7 ± 2.56^{a}	49.0 ± 2.35^{ab}
AsA (μmol g^{-1} DW)	38.9 ± 0.32^{b}	21.4 ± 0.25^{e}	27.8 ± 0.25^{d}	$33.4 \pm 3.0\ 6^{c}$	46.7 ± 4.67^{a}	36.9 ± 5.86^{c}
DHA (μmol g^{-1} DW)	7.6 ± 1.32^{d}	12.0 ± 0.14^{c}	16.6 ± 0.91^{b}	23.0 ± 3.75^{a}	9.0 ± 2.11^{cd}	15.2 ± 0.75^{b}
AsA/DHA	5.2 ± 0.94^{a}	1.8 ± 0.00^{b}	1.7 ± 0.13^{b}	1.5 ± 0.16^{b}	5.4 ± 1.77^{a}	2.2 ± 0.32^{b}

Each value is the mean of three replicates that were each analyzed in duplicate. For each parameter, the different letters indicate significant differences at $P \leq 0.05$, between both salt and period of treatment, as determined by Duncan's multiple range test

Table 3 Effect of 25 mM Na_2SO_4 or 50 mM NaCl on total glutathione (GSH + GSSG), reduced (GSH) glutathione, oxidized (GSSG) glutathione contents and reduced (GSH)/oxidized (GSSG) glutathione ratio in leaves of basil (*Ocimum basilicum* L.), cultivar Fine, after 15 and 30 days of treatment

	15 days			30 days		
	0 mM	25 mM Na_2SO_4	50 mM NaCl	0 mM	25 mM Na_2SO_4	50 mM NaCl
GSH + GSSG	0.45 ± 0.02^{c}	0.39 ± 0.07^{c}	0.75 ± 0.04^{a}	0.59 ± 0.10^{b}	0.62 ± 0.01^{b}	0.49 ± 0.12^{b}
GSH (μmol g^{-1} DW)	0.17 ± 0.01^{c}	0.30 ± 0.08^{b}	0.56 ± 0.04^{a}	0.36 ± 0.09^{b}	0.37 ± 0.03^{b}	0.30 ± 0.13^{b}
GSSG (μmol g^{-1} DW)	0.28 ± 0.09^{a}	0.09 ± 0.00^{c}	0.20 ± 0.01^{b}	0.23 ± 0.02^{b}	0.25 ± 0.03^{b}	0.19 ± 0.05^{b}
GSH/GSSG	0.58 ± 0.02^{c}	3.30 ± 0.94^{a}	2.84 ± 0.43^{a}	1.58 ± 0.34^{b}	1.54 ± 0.28^{b}	1.59 ± 0.82^{b}

Each value is the mean of three replicates that were each analyzed in duplicate. For each parameter, different letters indicate significant differences at $P \leq 0.05$, between both salt and period of treatment, as determined by Duncan's multiple range test

ROS have a dual function, i.e., they can cause damage and/or function as signaling molecules, as described in plant responses to several abiotic stresses (Miller et al. 2010; Frary et al. 2010). Hydrogen peroxide belongs to the most stable ROS and for this reason it is considered as one of the key diffusible signaling molecules. Recently, it has been shown that H_2O_2 produced by apoplastic polyamine oxidase influences the salinity stress signaling in tobacco and plays a role in balancing plant response between stress tolerance and cell death (Moschou et al. 2008). In the present study, no significant effect of Na_2SO_4 treatment on H_2O_2 production was registered. However, an increase in H_2O_2 content occurred in plants treated with NaCl (Table 1) after 15 days of treatment. Plants have evolved several cellular mechanisms to cope with these harmful ROS. The ascorbate–glutathione pathway takes part in this defense. Simultaneously, enzymes involved in the ascorbate–glutathione pathway (Fig. 2) and the leaf content of their metabolites (Tables 2, 3) were affected. The decrease in AsA/DHA ratio under both salt conditions, in Fine after 15 days of treatment, may lead to depletion in ascorbate pool, because the rate of AsA oxidation exceeds the capacity of the regenerative systems to reduce MDHA and DHA. As a consequence, in the absence of a de novo synthesis, a decrease in AsA could be monitored. The first step in the ascorbate–glutathione cycle is APX, which is the most important peroxidase involved in the detoxification of H_2O_2 (Noctor and Foyer 1998). The concomitant increase in H_2O_2 and APX activity observed under NaCl salinity during the first period suggested that, in this case, increased H_2O_2 content might be attributed to non-enzymatic reduction of superoxide by AsA or GSH, since the AsA content decreased (Table 2). This behavior suggests that under NaCl salinity, H_2O_2 detoxification rate is lower than that of its production. However, the constant APX activity and the decrease in POD activity noted under Na_2SO_4 salinity suggest that other enzymes may play a major role in H_2O_2 detoxification, which made H_2O_2 detoxification rate equal to that of its production under Na_2SO_4 salinity.

The main function of glutathione in the protection against oxidative stress is the reduction of DHA to AsA in the ascorbate–glutathione cycle (Noctor et al. 1998). The increase in total glutathione and the decrease in GSSG level observed in Fine cultivar under both salt treatments after 15 days determined an increase in GSH/GSSG ratio, suggesting an important role for this molecule in plant response to salt stress. Efficient recycling of glutathione is ensured by GR activity. The high relevant induction of GR activity found in leaves of salt-treated plants and the higher

GSH/GSSG ratio (Fig. 2b; Table 2) found after 15 days can be correlated with acclimation or even tolerance to the stress (Gossett et al. 1994). The latter may increase GR activity by an appearance of new GR isozymes (Alscher et al. 1997; Kocsy et al. 2001).

Peroxidases constitute a set of enzymes that catalyze the oxidation of substrates by H_2O_2 (Asada 1999). Therefore, decreased POD activity (Fig. 2c) observed under both salts and following 15 days of treatment seems to indicate that this enzyme does not take a crucial part in defense mechanisms against oxidative stress, or that suffering POD for salt toxicity, a co-operation is activated between different antioxidant enzymes to establish a proper H_2O_2 homeostasis.

Long-term effects of mild salt stress on growth and antioxidant systems

When salinity treatment was prolonged (30 days), leaf area and dry weight were suppressed similarly (Fig. 1a, b) and their number (Fig. 1c) remained unchanged under both 25 mM Na_2SO_4 and 50 mM NaCl salinities. This suggests that the Fine leaf area was decreased mainly through a reduction in individual leaf expansion rather than a decrease in new leaf production rate. These observations are consistent with the results of other reports under salinity stress (Cramer et al. 1994; D'Amico et al. 2003). The similar growth suppression of Fine cultivar under sodium chloride and sodium sulfate confirms its similar behavior to both salts as compared to Genovese that was previously shown to differ in its growth response depending on salinity type (Tarchoune et al. 2010). Under saline treatment, the deleterious effects of ROS on plants were made apparent through ion leakage and TBARS increases. Moreover, unchanged cell membrane stability and TBARS in *O. basilicum* cultivar Fine (Table 1) under Na_2SO_4 and NaCl salinities suggest that in both treatments, the activation of an efficient free radical scavenging system could minimize the adverse ROS effects as observed in several crops (Chaparzadeh et al. 2004; Pérez-López et al. 2009). Results from H_2O_2 content showed a significant increase in plants treated with NaCl; however, in those treated with Na_2SO_4, this parameter remained unchanged in comparison with the control (Table 1). In contrast to what was observed during the first period, after 30 days of treatment, both salts did not induce any effect on total ascorbate, and the higher AsA content observed with treatments in Fine was a symptom that AsA regeneration worked correctly. Higher AsA contents were correlated with a higher salt tolerance in different cultivars of watermelon (Yasar et al. 2006) and cotton (Gossett et al. 1994). The increased production in H_2O_2 following 30 days of treatment with NaCl could be due to decreased APX and POD activities (Fig. 2a, c). Na_2SO_4 did not significantly change the levels of H_2O_2, likely because of an induction of APX and POD activities (Table 1; Fig. 2a, c). However, no difference between salt treatments was detected for total glutathione, GSH and GSSG contents, GSH/GSSG ratio (Table 3) or for GR activity (Fig. 2b). This indicates that glutathione metabolism is unaffected despite the changes observed in the first period and in the AsA–GSH cycle as proved by the activities of the other enzymes of the cycle.

As a whole, our data suggest that the enhancement of the antioxidative response is of crucial significance for *O. basilicum* (cv. Fine) grown under Na_2SO_4 or NaCl salinity. However, the different activities of antioxidant molecules and enzymes in this cultivar suggest a different kind of reaction to the two salts. Therefore, the different pattern of antioxidative response noted under Na_2SO_4 and NaCl salinity is difficult to attribute to changes in Na^+ and water contents, given that no differences in Na^+ levels or in water content were observed between the two salts (non shown data). We speculate that neither Na^+ levels nor water content can explain the different antioxidative responses to these salts, supporting the hypothesis that Na^+ and water contents remain an important factor influencing antioxidative response, but other mechanisms may be involved in our case.

In summary, two main conclusions can be drawn from these findings. First, similar effects of NaCl or Na_2SO_4 salinity on growth response in *O. Basilicum* (cv. Fine) after both periods of treatment (15 and 30 days) were observed with no differences in Fine growth activity after 15 days of treatment. Similar growth reductions were noted, however, after 30 days of treatment. Second, analyzing the antioxidant metabolism of Fine cultivar under either Na_2SO_4 or NaCl, a different kind of response to stress was observed and several defense strategies were activated. The lower levels of oxidative stress marker observed under Na_2SO_4 in comparison with NaCl suggest that under the former salt, Fine cultivar had a better capacity to cope with oxidative stress thanks to a higher GR activity and a capacity to increase GSH in the first period of treatment, even if there was an induction of APX and POD activities and AsA level at the second one.

Author contributions Imen Tarchoune performed the experiments for obtaining the analytical data, interpreted the results and wrote the manuscript, besides revising the manuscript after each review. Cristina Sgherri developed the experimental methods, helped to do some experiments and contributed to the setting of the manuscript as well as to its correction. Mokhtar Lachaâl and Riccardo Izzo, scientific and administrative directors of the two laboratories where the research work was conducted supported the work financially. Flavia Navari-Izzo, the designer of the

experiments, supervised their progress and contributed by scientific advices during manipulation and correction of the manuscript. Zeineb Ouerghi provided scientific direction and supervision to the whole research work. She contributed to the final revision of the manuscript.

Acknowledgments This work was supported by the University of Pisa (Fondi di Ateneo 2007–2008). Imen Tarchoune staying at the University of Pisa was supported by the Tunisian Ministry of Higher Education, Scientific Research, and Technology.

References

Ahmad P, Sarwat M, Sharma S (2008) Reactive oxygen species, antioxidants and signalling in plants. J Plant Biol 51:167–173

Alscher RG, Donahue JL, Cramer CL (1997) Reactive oxygen species and antioxidants: relationships in green cells. Physiol Plant 100:224–233

Alscher RG, Erturk N, Heath LS (2002) Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. J Exp Bot 53:1331–1341

Anderson ME (1985) Determination of glutathione and glutathione disulfide in biological samples. Methods Enzymol 113:548–555

Anderson JV, Chevone BI, Hess JL (1992) Seasonal variation in the antioxidant system of eastern white pine needles. Plant Physiol 98:501–508

Asada K (1999) The water–water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. Ann Rev Plant Physiol Plant Mol Biol 50:601–639

Asada K (2006) Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiol 141:391–396

Attia H, Ouhibi C, Ellili A, Msilini N, Bouzaien G, Karray N, Lachaâl M (2010) Analysis of salinity effects on basil leaf surface area, photosynthetic activity, and growth. Acta Physiol Plant. doi: 10.1007/s11738-010-0607-6

Cavalcanti FR, Oliveira JTA, Martins MAS, Viegas 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) Antioxidative responses of *Calendula Officinalis* under salinity conditions. Plant Physiol Biochem 42:695–701

Cramer GR, Alberico GJ, Schmidt C (1994) Leaf expansion limits dry matter accumulation of salt-stressed maize. Aust J Plant Physiol 21:663–674

D'Amico ML, Izzo R, Tognoni F, Pardossi A, Navari-Izzo F (2003) Application of diluted sea water to soilless culture of tomato (*Lycopersicon esculentum* Mill.): effects on plant growth, yield, fruit quality and antioxidant capacity. Food Agri Environ 1:112–116

Demmig-Adams B, Adams WW III (1994) Light stress and photoprotection related to the xanthophyll cycle. In: Foyer CH, Mullineaux PM (eds) Causes of photooxidative stress and amelioration of defence systems in plants. CRC Press, Boca Raton, pp 105–126

Foyer C (1997) Oxygen metabolism and electron transport in photosynthesis. In: Scandalios G (ed) Oxidative stress and the molecular biology of antioxidant defence. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp 687–721

Frary A, Göl D, Keles D, Ökmen B, Pmar H, Sigva H, Yemenicioğlu A, Doğanlar S (2010) Salt tolerance in *Solanum pennellii*: antioxidant response and related QTL. BMC. Plant Biol 10–58

Fryer MJ (1992) The antioxidant effects of thylakoid vitamin E (α-tocopherol). Plant Cell Environ 15:381–392

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

Gupta KJ, Stoimenova M, Kaiser WM (2005) In higher plants, only root mitochondria, but not leaf mitochondria reduce nitrite to NO, in vitro and in situ. J Exp Bot 56:2601–2609

Hideg E (1999) Free radical production in photosynthesis under stress conditions. In: Pessarakli M (ed) Handbook of plant and crop stress. Marcel Dekker, New York, pp 911–930

Hodges DM, Delong JM, Forney CF, Prange RK (1999) Improving the thiobarbituric acid reactive substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta 207:604–611

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

Kocsy G, Galiba G, Brunold C (2001) Role of glutathione in adaptation and signalling during chilling and cold acclimation in plants. Physiol Plant 113:158–164

Lin CC, Kao CH (2002) Osmotic stress-induced changes in cell wall peroxidase activity and hydrogen peroxide level in roots of rice seedlings. Plant Growth Regul 37:177–183

Manchanda G, Garg N (2008) Salinity and its effect on the functional biology of legumes. Acta Physiol Plant 30:595–618

Miller G, Suzuki N, Ciftci-Yilmazi N, Mittler R (2010) Reactive oxygen species homeostasis and signalling during drought and salinity stresses. Plant Cell Environ 33:453–467

Moschou PN, Paschalidis KA, Delis ID, Andriopoulou AH, Lagiotis GD, Yakoumakis DI, Roubelakis-Angelakis KA (2008) Spermidine exodus and oxidation in the apoplast induced by abiotic stress is responsible for H_2O_2 signatures that direct tolerance responses in tobacco. Plant Cell 20:1708–1724

Munns R (2002) Comparative physiology of salt and water stress. Plant Cell Environ 25:239–250

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

Noctor G, Arisi ACM, Jouanin L, Foyer CH (1998) Manipulation of glutathione and amino acid biosynthesis in the chloroplast. Plant Physiol 118:471–482

Pérez-López U, Robredo A, Lacuesta M, Sgherri C, Muñoz-Rueda A, Navari- Izzo F, Mena-Petite A (2009) The oxidative stress caused by salinity in two barley cultivars is mitigated by elevated CO_2. Physiol Plant 135:29–42

Pérez-López U, Robredo A, Lacuesta M, Sgherri C, Mena-Petite A, Navari-Izzo F, Muñoz-Rueda A (2010) Lipoic acid and redox status in barley plants subjected to salinity and elevated CO_2. Physiol Plant 139:256–268

Quartacci MF, GlišIč O, Stevanovič B, Navari-Izzo F (2002) Plasma membrane lipids in the resurrection plant *Ramonda serbica* following dehydration and rehydration. J Exp Bot 53:2159–2166

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

Sgherri CLM, Loggini B, Puliga S, Navari-Izzo F (1994) Antioxidant system in *Sporobolus stapfianus*: change in response to desiccation and rehydration. Phytochem 35:561–565

Sgherri CLM, Navari-Izzo F, Pardossi A, Soressi GP, Izzo R (2007) The influence of diluted seawater and ripening stage on the content of antioxidants in fruits of different tomato genotypes. J Agri Food Chem 55:2452–2458

Smith IK, Vierheller TL, Thorne CA (1988) Assay of glutathione reductase in crude tissue homogenates using 5,5′-dithiobis (2-nitrobenzoic acid). Anal Biochem 175:408–413

Tarchoune I, Sgherri C, Izzo R, Lachaâl M, Ouerghi Z, Navari-Izzo F (2010) Antioxidative responses of *Ocimum basilicum* to sodium chloride or sodium sulphate salinization. Plant Physiol Biochem 48:772–777

Wang SY, Jiao HJ, Faust M (1991) Changes in ascorbate, glutathione, and related enzymes activities during thidiazuron-induced bud break of apple. Physiol Plant 82:231–236

Yasar F, Kusvuran S, Ellialtioglu S (2006) Determination of antioxidant activities in some melon (*Cucumis melo* L.) varieties and cultivars under salt stress. J Hortic Sci Biotech 81:627–630