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Effect of Water Deficit and Salt Stress on Oxidative Parameters and Antioxidant Systems in Tomato (*Solanum lycopersicum* L.) Fruits

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ABSTRACT

In this study, the differential behaviour of antioxidant systems between water stress and salt stress, considering two stages of development of the fruit was investigated. The activities of superoxide dismutase (SOD) and catalase (CAT), as well as the activities and the relative transcript levels of the enzymes of ascorbate-glutathione cycle: ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR) and glutathione reductase (GR), the levels of ascorbate pool and hydrogen peroxide (H_2O_2) and malonyldialdehyde (MDA) contents were studied in fruits of tomato plants (*Solanum lycopersicum* L. cv. 'Micro-Tom') subjected to water and salt stresses. Water deficit was induced by holding off the irrigation for 3 and 6 days. To apply salt stress, plants were treated with 100 mM of NaCl for 3 and 6 days. H_2O_2 content was generally increased with both water and salt stresses, however, MDA content was increased only with salt stress. Changes in ascorbate pool were noted in plants subjected to salt stress more than in plants subjected to water stress. Moreover, changes in SOD and CAT activities and in DHAR, MDHAR, APX and GR activities and relative transcript levels were depending on the type and period of stress and the fruit development stage. Moreover, the changes in enzyme activities in response to stress were not directly related to changes in the corresponding gene expressions. These results suggest that water and salt stress lead to oxidative stress and modulates the antioxidative responses of tomato fruits.

Keywords: antioxidant enzymes, ascorbate, gene expression, oxidative stress
Abbreviations: APX, ascorbate peroxidase; **AsA**, ascorbate; **CAT**, catalase; **DAF**, days after flowering; **DHA**, dehydroascorbate; **DHAR**, dehydroascorbate reductase; **GR**, glutathione reductase; **GSH**, reduced glutathione; **GSSG**, oxidised glutathione; **MDA**, malonyldialdehyde; **MDHAR**, monodehydroascorbate reductase; **ROS**, reactive oxygen species; **SOD**, superoxide dismutase

INTRODUCTION

Plants experience a multitude of stress conditions, which are broadly classified into two categories: biotic and abiotic (Grover *et al.* 1998). Among the abiotic stresses, drought and salt stress are the most important factors influencing the productivity of crops (Wild 2003; Rengasamy 2006). Plant responses to drought and salt stress have much in common. Salinity reduces the ability of plants to take up water, and this quickly causes reductions in growth rate, along with a suite of metabolic changes identical to those caused by drought. Drought and salt stress have detrimental effects on plant growth, development processes, integrity of cellular membranes and the functioning of the plant photosynthetic apparatus (Sairam and Tyagi 2004). They also induce several physiological, biochemical and molecular responses in plants.

It is well documented that drought and salt stress exert at least in part of their effects by causing oxidative damage. This damage is caused by increased production of reactive oxygen species (ROS) (Smimoff 1995). ROS like superoxide radical ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) are routinely generated during normal plant metabolic processes. The excess production of ROS during drought and salt stress results from impaired electron transport processes in chloroplast and mitochondria as well as from pathways such as photorespiration. In the absence of a protective mechanism in plants, ROS can cause serious damage to different aspects of cell structure and function such as initiating lipid peroxidation and damaging DNA, proteins and other small molecules (Arora *et al.* 2002). Fortunately, plants have developed various protective mechanisms to eliminate or reduce ROS. Two types of antioxidants, enzymatic and non-enzymatic, have been shown to be involved in scavenging of ROS in plants. The antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR), have been reported to be implicated in scavenging of ROS under stress conditions (Sairam and Srivastava 2002). SOD catalyzes the dismutation of superoxide to H_2O_2 (Bowler *et al.* 1992). However, H_2O_2 is also toxic to the cells and has to be further scavenged by CAT or APX or both, to water and oxygen (Zhu *et al.* 2004; Sairam *et al.* 2005). In the ascorbate-glutathione cycle, APX reduces H_2O_2 using ascorbate (AsA) as an electron donor. The first product of enzymatic action of APX, namely monodehydroascorbate (MDHA), is reduced to AsA by MDHAR. MDHA that escapes this route of re-cycling rapidly disproportionates to dehydroascorbate (DHA) (Foyer *et al.* 1994). DHA is converted back to AsA by the action of DHAR. Reduced glutathione (GSH) functions as an electron donor for DHAR, oxidized glutathione (GSSG) produced in DHA reduction is reconverted to GSH by GR (Mittler 2002; Apel and Hirt 2004). The second type is non-enzymatic antioxidants which consist of antioxidant molecules such as α-tocopherol, carotenoids, glutathione and ascorbate play a key role in scavenging free radicals in plants (Hernàndez *et al.* 2000; Verma and Mishra 2005). AsA also acts as a secondary antioxidant during reductive recycling of the oxidized form of α-tocopherol (Noctor and Foyer 1998; Smirnoff and Wheeler 2000).

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Original Research Paper

Tomato is the most consumed fruit in the world and its consumption is increasing steadily each year (FAO 2007), and it has been suggested that the consumption of tomatoes, which have high levels of antioxidants, may have a strong positive impact on public health by decreasing the risk of cardiovascular diseases (Passam and Karapanos 2008). Tomato plants are highly susceptible to water and salt stresses notably during growth phase (Dodds *et al.* 1997). In tomato plants, the changes in the antioxidants systems in response to different stresses have been investigated in detail in leaves, and there is a considerable amount of literature concerning leaf responses to drought and salt stress with respect to oxidative stress defence mechanisms. However, little is known about such response mechanisms in fleshy fruits subjected to these stresses. Pericarp of tomato fruits initially contains chloroplasts that are photosynthetically active, but they differentiate to non-photosynthetic chromoplasts during the ripening process. So, depending on the age of fruits, chloroplast photosynthesis or mitochondria respiration is a primary source of superoxide (Purvis *et al.* 1995).

Understanding how plants respond to drought and salt stresses can play a major role in stabilizing crop performance under drought and saline conditions. In the present study, attempts were made to examine the differential behaviour of antioxidant systems in tomato fruits subjected, at the medium and the end of expansion cell fruit phase, to drought and to saline treatment. To this end, we investigated changes in the activities of antioxidant enzymes (SOD and CAT) as well as in the activities and the relative transcript levels of the enzymes of ascorbate-glutathione cycle (APX, MDHAR, DHAR and GR) in fruits of tomato plants exposed to drought and salt stress. In addition, the contents of some non-enzymatic antioxidants, such as AsA and DHA were also assessed. Levels of MDA and H_2O_2 were measured as oxidative damage indicators.

MATERIALS AND METHODS

All chemicals and reagents used in this experiment were purchased from Sigma-Aldrich (France) except that marked in the text

Plant material and treatments

Tomato (*Solanum lycopersicum* L. cv. 'Micro-Tom') seeds were obtained from GAFL (INRA Avignon, France) and germinated in boxes filled with peat. When the first real leaf appeared, seedlings were transplanted into 4 L plastic containers containing a mixture of peat and vermiculite (1: 1). From germination until the end of the experiment, the plants were grown in the same growth room illuminated with cool-white fluorescent lamps under a 16/8 h light/dark cycle, a photosynthetic photon flux density (PPPD) of 300 µmol. m^{-2}. s^{-1}, a relative humidity of 60 ± 5% and day/night temperature of 25/20°C.

The plants were irrigated twice a week with a nutrient solution containing: 8 µM $MnCl_2$, 0.5 µM $CuSO_4 \cdot 5H_2O$, 1.4 µM $ZnSO_4$, 46 µM H_3BO_3, 0.25 µM $Na_2MoO_4 \cdot 2H_2O$, 4.1 mM KNO_3, 3.4 mM $Ca(NO_3)_2 \cdot 4H_2O$, 0.9 mM K_2SO_4, 1 mM $MgSO_4 \cdot 7H_2O$ and 1.5 mM KH_2PO_4 (Sonnefeld and Van der Wees 1984). The iron source was Fe-EDDHA chelate (0.6%). On the other days of week plants were irrigated with water.

At the time of anthesis, flowers were tagged for determining fruit age. Plants were grouped into three sets: First set (C), control plants were irrigated daily with an amount of water equal to the evapotranspired water. Second set (WS), plants subjected to water stress by holding off the irrigation. Third set (SS), plants subjected to salt stress by treatment with 100 mM of NaCl. The fruits were harvested after 3, and 6 days of treatment, between 9 and 10 a.m. and they were grouped following fruit age into small green fruits (SG fruits; fruit age was 25 days after flowering; DAF) or mature green fruits (MG fruits; fully developed green fruit, fruit age was 40 DAF). For each stage and treatment, fruits were harvested from three plants and immediately frozen in liquid nitrogen, ground and the resulting powder was stored at -80°C.

Leaf and fruit water parameters

Fruit water potential ($F\Psi_w$) was measured using a water potential system (PSΨPRO™) attached to C-52 chambers (Wescor Inc, Logan, Utah, USA). Fruit osmotic potential ($F\Psi_O$) was measured using the NanoOsmometer (VAPRO, Vapor Pressure Osmometer, WESCOR). Fresh weight (FW) of fruits was determined just after harvesting. The dry weight (DW) was measured after that fresh material was dried at 70°C for 72 h. The water content was determined as:

$$WC = [FW\text{-}DW]/FW \times 100.$$

Determination of H_2O_2 content

Hydrogen peroxide levels were determined as described by Murshed *et al.* (2008a). Frozen powder of fruits (0.25 g) was homogenized in an ice bath with 1 mL 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 12,000 × *g* for 15 min at 4°C. Aliquots of 100 µL from each tube were placed in 96-well plates and 50 µL of 10 mM potassium phosphate buffer (pH 7.0) and 100 µL of 1 M KI were added in each well. Commercial H_2O_2 was used to generate a standard curve. The plate was briefly vortexed and the absorbance readings were taken at 390 nm in a micro-plate reader (PowerWave HT Microplate Spectrophotometer from BioTek, the same micro-plate reader was used for all the experiments listed below). The content of H_2O_2 was determined using the standard curve.

Determination of the malonyldialdehyde content

For the measurement of lipid peroxidation in fruits, the thiobarbituric acid (TBA) test, which determines malonyldialdehyde (MDA) as an end product of lipid peroxidation (Murshed *et al.* 2008a), was used. Frozen fruit powder (0.25 g) was homogenized in 1 mL 0.1% (w/v) TCA solution. The homogenate was centrifuged at 12,000 × *g* for 15 min and 0.5 mL of the supernatant was added to 1 mL 0.5% (w/v) TBA in 20% TCA. The mixture was incubated in boiling water for 30 min, and the reaction stopped by placing the reaction tubes in an ice bath. Tubes were briefly vortexes and triplicate, 200 µL aliquots from each tube were placed in 96-well plates, and the absorbance of supernatant was read at 532 nm in a micro-plate reader. The value for non-specific absorption at 600 nm was subtracted. The amount of MDA–TBA complex (red pigment) was calculated from the extinction coefficient 155 $mM^{-1}.cm^{-1}$.

Determination of ascorbate and dehydroascorbate contents

Total ascorbate (AsA plus DHA) and AsA contents were measured according to Kampfenkel *et al.* (1995), scaled down for microplates by Murshed *et al.* (2008a). A 0.5 g sample of frozen powder of fruits was homogenized in 1 mL of cold 6% (w/v) TCA. The homogenate was centrifuged at 16,000 × *g* at 4 °C for 15 min. The supernatant was used for total ascorbate and AsA determination.

For measurements of total ascorbate, 10 µL of extract were added to 10 µL of 10 mM DL-dithiothreitol (DTT) and 20 µL of 0.2 mM phosphate buffer (pH 7.4). After incubation for 15 min at 42°C, 10 µL of 0.5% (w/v) *N*-ethylmaleimide (NEM) was added with incubation for 1 min at room temperature to remove excess DTT. This was followed by adding 150 µL of a reagent prepared just before use by mixing 50 µL of 10% TCA, 40 µL of 42% (v/v) of orthophosphoric acid (H_3PO_4), 40 µL of 4% (w/v) 2.2-bipyridyl dissolved in ethanol (70%) and 20 µL of 3% (w/v) ferric chloride. After further incubation for 40 min at 42°C, the absorbance was measured at 525 nm in a micro-plate reader. For AsA determination, the same reaction was used but 0.2 M phosphate buffer (pH 7.4) was used in place of DTT and NEM. The amount of DHA was estimated from the difference of total ascorbate and AsA. Commercial L-Ascorbic acid was used to generate a standard curve.

Table 1 Sets of primers used to amplify gene-specific regions, corresponding size and accession number of the amplified product.

PCR fragment	Encoded protein	Primer sequence (5′ → 3′)	Size (bp)	Accession number
SlAPXt	Thylakoid-bound ascorbate peroxidase	Sense: TTCACCCAATGACTTCCCT Antisense: TATCATTTAGTCCCATTCTGT	699	FJ532352
SlAPXcyto	Cytosolic ascorbate peroxidase	Sense: GTTGAAGGTCGCTTGCCG Antisense: CCAAGGTATGGGCACCAG	118	FJ532353
SlDHAR1	Dehydroascorbate reductase	Sense: TGCCTCTGTGGGCTCGAA Antisense: ACCACCCTGCGATGACGT	335	FJ532354
SlMDHAR	Monodehydroascorbate reductase	Sense: GGAGAAGTTTCGTTGCTGCT Antisense: TGAGCAGCTTTCCTGAATTGT	371	FJ544908
SlGRcp	Chloroplastic glutathione reductase	Sense: TGGAGGCGTCGGTGGCAC Antisense: CTATAGCATACTCGCTTCCAG	362	FJ544906
SlGRcyto	Cytosolic glutathione reductase	Sense: GCAAAGAATTATGGATGGGA Antisense: CACAGCACGCTTTGGTAA	333	FJ544907
SlActin	Actin	Sense: ATGACTCAAATCATGTTTGAG Antisense: TACCTTAATCTTCATGCTGCT	633	FJ532351

Antioxidant enzyme assays

1. Extraction of enzymes

Proteins extraction was performed according to Murshed *et al.* (2008b). Frozen fruits powder (0.20 to 0.4 g) homogenizes in 1 ml of 50 mM MES/KOH buffer (pH 6.0), containing: 40 mM KCl, 2 mM $CaCl_2$, and 1 mM AsA. Extracts were centrifuged at 4°C for 15 min at 16,000 × *g*, and the supernatants were analysed immediately for enzyme activities. Protein was quantified by Bradford's method (Bradford 1976).

2. Enzyme assays

All enzyme activities were determined in 200 µL volume kinetic reactions at 25°C, using a micro-plate reader. APX, DHAR, MDHAR and GR activities were measured by the method of Murshed *et al.* (2008b). SOD activity was assayed in a 1.0 ml reaction mixture containing 50 mM potassium phosphate (pH 7.8) buffer, 13 mM methionine, 75 µM nitro blue tetrazolium (NBT), 0.1 mM EDTA, 10 µL of sample supernatant and 2 µM riboflavin. Tubes were briefly vortexes and triplicate, 200 µL aliquots from each tube, were placed in plastic 96-well plates. Commercial SOD in amounts of 0.6, 1.2, 1.8, 2.4 and 3 units was used to generate a standard curve. The plates were then placed under white light provided by a commercial overhead-transparency projector (Horizon, Model Apollo, Lincolnshire, IL, USA) with a PPPD of 500 µmol. m^{-2}. s^{-1} for 5 min. After the light treatment, absorbance readings were taken at 560 nm in a plate reader. SOD in the extract inhibited the photochemical reduction of NBT to blue formazan. Activity was expressed in units of SOD from the standard curve of activity units versus absorbance. This method was modified from that of Dhindsa *et al.* (1981). CAT activity was measured using a method adapted from that of Aebi (1984) in a reaction mixture containing 50 mM phosphate buffer (pH 7.0), 15 mM H_2O_2 and 20 µL of sample supernatant. Activity was determined by measuring the decrease in the reaction rate at 240 nm and calculated using a 43.6 M^{-1}. cm^{-1} extinction coefficient.

RNA extraction, cDNA synthesis and quantitative RT-PCR detection

Total RNA was isolated from 50-100 mg of frozen fruits powder using Tri Reagent (MRC, Molecular Research Center, INC) according to manufacturer's instructions. RNA were quantified spectrophotometrically measuring the absorbance at 260 and 280 nm. RNA integrity was verified on 1% agarose gels.

For RT-PCR experiments, first-strand cDNA was synthesized from 4 µg of total RNA using the Masterscript™ RT-PCR system (5 PRIME) according to manufacturer's instructions. A first step was used for initial template RNA denaturation at 65°C for 5 min. In the second step, the first-strand cDNA was synthesized using Oligo d(*T*) primer at 42°C for 60 min, and then the reaction was interrupted by enzyme inactivation at 85°C for 5 min.

The quantitative assessment of mRNA levels was performed using the RealMasterMix SYBR ROX (5 PRIME) in Mastercycler ep Realplex (Eppendorf). The quantification of the accumulation of the target transcript relative to the actin (*SlActin*) transcript, taken as the control gene for the experimental conditions, was performed with specific primers for each gene. Specific primers pairs of *SlActin*, *SlAPXcyto*, *SlAPXt*, *SlDHAR1*, *SlDHAR2*, *SlMDHAR*, *SlGRcyto* and *SlGRcp* were designed based on sequences present in the GenBank database (http://www.ncbi.nlm.nih.gov) using DNAMAN software (**Table 1**). Three independent RNA isolations, with two replications for each of the RNA isolations, were performed for each stage and treatment. Amplified fragments of PCR product were purified using the QIAquick PCR Purification Kit (QIAGEN) following manufacturer's instructions, and sequenced using Genome Express services. Sequences data have been deposited with the GenBank database under accession numbers referenced in **Table 1**.

Statistic analysis

The experiment was repeated three times, and the values presented are the means of the three experiments. The results are expressed as means with standard error (± SE). Statistical significance was determined by the Fisher test (LSD) ($p<0.05$) using MINITAB Release 14 Statistical Software.

RESULTS

Fruit water parameters

The effects of water and salt stresses on fruit water potential ($F\Psi_w$), fruit osmotic potential ($F\Psi_O$), turgor potential ($F\Psi_t$) and water content of fruits (FWC) are shown in **Table 2**. Under water stress, $F\Psi_w$ and $F\Psi_t$ decreased in SG and MG fruits, while $F\Psi_O$ not affected except in MG fruits of plants stressed for 6 days, where it decreased. Under salt stress, $F\Psi_w$, $F\Psi_O$ and $F\Psi_t$ decreased in all fruits of plants stressed except in MG fruits of plants stressed for 6 days, where $F\Psi_w$ not changed and $F\Psi_t$ increased. FWC was not changed by water or salt stresses (**Table 2**).

H_2O_2 content and lipid peroxidation:

The effects of water and salt stresses on H_2O_2 and malonyldialdehyde (MDA) contents are shown in **Table 3**. In control conditions, H_2O_2 content was more elevated in SG fruits than in MG fruits. Water deficit treatment induced an increase in H_2O_2 content in SG and MG fruits, about 250 and 180%, respectively. With salt stress treatments, H_2O_2 content increased only in SG fruits, but it decreased in MG fruits (**Table 3**). MDA content, used as indicator of membrane lipid peroxidation damages, was affected by water stress only in SG fruits stressed for 3 days, where it increased about 50%. Salt stress did not affect MDA content in SG fruits; however, it increased the MDA content in MG fruits, the increase was 36% in fruits stressed for 3 days and 50% in fruits stressed for 6 days (**Table 3**).

Table 2 Fruit water potential ($F\Psi_w$; $MPa_)$, fruit osmotic potential ($F\Psi_O$; MPa), turgor pressure ($F\Psi_t$; MPa) and water content of fruits (FWC; %) of control plants (C) and plants subjected to water or salt stress (S) for three and six days (3D and 6D). Values are the mean (± S.E.) of five replicates, and different letters within lines indicate significant differences determined by the Fisher test (LSD) (P<0.05). SG: SG: Fruits at small green stage, MG: Fruits at mature green stage.

		3D			6D		
		C	Water stress	Salt stress	C	Water stress	Salt stress
$F\Psi_w$	SG	- 0.37 ± 0.01 a	- 0.67 ± 0.03 b	- 0.63 ± 0.02 b	- 0.36 ± 0.02 a	- 0.58 ± 0.03 b	- 0.54 ± 0.05 b
	MG	- 0.44 ± 0.01 a	- 0.58 ± 0.01 b	- 0.61 ± 0.03 b	- 0.43 ± 0.03 a	- 0.85 ± 0.04 b	- 0.44 ± 0.03 a
$F\Psi_O$	SG	- 0.71 ± 0.02 a	- 0.71 ± 0.03 a	- 0.78 ± 0.02 b	- 0.71 ± 0.02 a	- 0.75 ± 0.03 a	- 0.81 ± 0.04 b
	MG	- 0.72 ± 0.03 a	- 0.78 ± 0.04 a	- 0.82 ± 0.04 b	- 0.72 ± 0.02 a	- 0.95 ± 0.06 b	- 0.92 ± 0.05 b
$F\Psi_t$	SG	0.34 ± 0.01 a	0.04 ± 0.01 b	0.15 ± 0.01 c	0.35 ± 0.01 a	0.17 ± 0.01 b	0.27 ± 0.01 c
	MG	0.28 ± 0.02 a	0.20 ± 0.03 b	0.21 ± 0.01 b	0.29 ± 0.01 a	0.10 ± 0.02 b	0.48 ± 0.02 c
FWC	SG	90.42 ± 0.25 a	89.70 ± 0.4 a	89.64 ± 0.44 a	90.62 ± 0.34 a	90.11 ± 0.37 a	89.48 ± 0.22 a
	MG	90.42 ± 0.35 a	90.13 ± 0.40 a	89.83 ± 0.33a	90.50 ± 0.24 a	89.72 ± 0.31 a	89.81 ± 0.31a

Table 3 H_2O_2 (nmol.g^{-1} FW) and malonyldialdehyde (MDA; nmol.g^{-1} FW) contents in fruits of control plants (C) and in fruits of plants subjected to water or salt stresses (S) for three and six days (3D and 6D). Values are the mean (± S.E.) of five replicates, and different letters within columns indicate significant differences from the control value determined by the Fisher test (LSD) (P<0.05). SG: Fruits at small green stage, MG: Fruits at mature green stage.

		3D			6D		
		C	Water stress	Salt stress	C	Water stress	Salt stress
H_2O_2	SG	291.39 ± 31.03 a	731.90 ± 54.33 b	785.25 ± 27.29 b	291.40 ± 31.10 a	541.90 ± 63.65 b	371.51 ± 26.55 c
	MG	40.87 ± 1.73 a	102.37 ± 2.01 b	14.85 ± 0.33 c	40.72 ± 1.63 a	137.86 ± 3.66 b	13.72 ± 0.33 c
MDA	SG	28.15 ± 2.32 a	50.68 ± 4.41 b	36.57 ± 3.32 c	28.38 ± 2.34 a	33.53 ± 4.12 a	32.73 ± 2.35 a
	MG	28.62 ± 2.17 a	27.47 ± 2.32 a	44.46 ± 3.28 b	28.53 ± 2.64 a	27.50 ± 2.72 a	48.42 ± 1.89 b

Table 4 Ascorbate (AsA) and dehydroascorbate (DHA) concentrations (nmol.g^{-1} FW) and redox state (AsA/AsA+DHA) in fruits of control plants (C) and in fruits of plants subjected to water or salt stresses (S) for three and six days (3D and 6D). Values are the mean (± S.E.) of five replicates, and different letters within columns indicate significant differences from the control value determined by the Fisher test (LSD) (P<0.05). SG: Fruits at small green stage, MG: Fruits at mature green stage.

		3D			6D		
		C	Water stress	Salt stress	C	Water stress	Salt stress
AsA	SG	10.36 ± 1.01 a	8.77 ± 0.70 a	10.75 ± 1.13 a	10.34 ± 1.00 a	9.57 ± 0.89 a	13.78 ± 1.09 b
	MG	17.37 ± 1.12 a	19.14 ± 0.83 a	22.23 ± 0.53 b	17.35 ± 1.10 a	18.16 ± 1.23 a	26.02 ± 1.33 b
DHA	SG	7.97 ± 0.61 a	8.30 ± 0.26 a	9.79 ± 0.11 b	7.96 ± 0.60 a	10.42 ± 0.33 b	5.86 ± 0.38 c
	MG	5.20 ± 0.62 a	4.56 ± 0.51a	2.38 ± 0.67 b	5.22 ± 0.63 a	4.70 ± 0.70 a	6.61 ± 0.83 a
Redox state	SG	0.57 ± 0.03 a	0.51 ± 0.05 a	0.52 ± 0.06 a	0.57 ± 0.03 a	0.48 ± 0.02 b	0.70 ± 0.05 b
	MG	0.77 ± 0.05 a	0.81 ± 0.06 a	0.90 ± 0.05 b	0.77 ± 0.05 a	0.79 ± 0.06 a	0.80 ± 0.07 a

Ascorbate and dehydroascorbate contents

The effects of water deficit and salt stress on ascorbate (AsA) and dehydroascorbate (DHA) concentrations and the ascorbate redox state (AsA/AsA+DHA) in fruits are shown in **Table 4**. Without stress treatment, AsA concentration and AsA redox state were less elevated in SG fruits than in MG fruits; in contrast, DHA concentration was more elevated in SG fruits (**Table 4**). Water stress treatment, have not effect on AsA levels in SG and MG fruits. However, AsA concentration increased about 43% in SG fruits of plants subjected to salt stress for 6 days and about 43 and 75% in MG fruits of plants subjected to salt stress for 3 and 6 days, respectively (**Table 4**). DHA content was not altered by water stress except in SG fruits of plants stressed for 6 days, where it increased about 20%. Under salt treatments, DHA concentration increased in SG fruits of plants stressed for 3 days but it decreased about 44% in fruits of plants stressed for 6 days. It decreased also in MG fruits stressed for 3 days (**Table 4**). Ascorbate redox state was not affected by water and salt stresses except in SG fruits stressed for 6 days, where it decreased under water stress and increased under salt stress. Ascorbate redox state increased also in MG fruits subjected to salt stress for 3 days (**Table 4**).

Activities of antioxidant enzymes

Activities of antioxidant enzymes (SOD, CAT, APX, MDHAR, DHAR and GR) were determined in the fruits of plants subjected to water and salt stresses and at two different development stages (SG and MG) (**Fig. 1**).

Under water stress, SOD activity was increased in SG and MG fruits of plants stressed for 3 days but it not changed in fruits of plants stressed for 6 days (**Fig. 1A**). CAT activity increased in SG and MG fruits (268 and 182%, respectively) of plants stressed for 3 days, and only in MG fruits (176%) of plants stressed for 6 days (**Fig. 1B**). APX activity was increased in fruits at both development stages by all water stress treatments (**Fig. 1C**). MDHAR activity increased in MG fruits of plants stressed for 3 and 6 days (136 and 345%, respectively); however, it decreased in SG fruits (**Fig. 1D**). DHAR activity increased only in MG fruits stressed for 3 and 6 days (206 and 273%, respectively) (**Fig. 1E**). GR activity increased only in MG fruits of plants stressed for 3 and 6 days (337 and 146%, respectively) (**Fig. 1F**).

Under salt stress, SOD activity increased in all fruits of plants stressed except in SG fruits of plants stressed for 3 days, where it not affected (**Fig. 1A**). CAT activity increased about 251% in SG fruits of plants stressed for 3 days and about 295 and 138% in SG and MG fruits of plants stressed for 6 days, respectively (**Fig. 1B**). APX and MDHAR activities were increased in SG and MG fruits by all salt stress treatments (**Fig. 1C**, **Fig. 1D**). DHAR activity decreased in SG fruits of plants stressed for 3 days; while it was increased in MG fruits by all salt stress treatments (**Fig. 1E**). Moreover, GR activity increased in SG fruits of plants stressed for 6 days (439%), but it was decreased in MG fruits by all salt stress treatments (**Fig. 1F**).

Gene expressions of antioxidant enzymes

The relative transcript levels of some genes encoding the enzymes associated with the ascorbate-glutathione cycle: cytosolic *APX* (*SlAPXcyto*), thylakoid-bound *APX* (*SlAPXt*), *SlMDHAR*, *SlDHAR1*, cytosolic *GR* (*SlGRcyto*) and chloroplastic *GR* (*SlGRcp*) were measured in fruits of control and treated plants using real-time quantitative RT-PCR and nor-

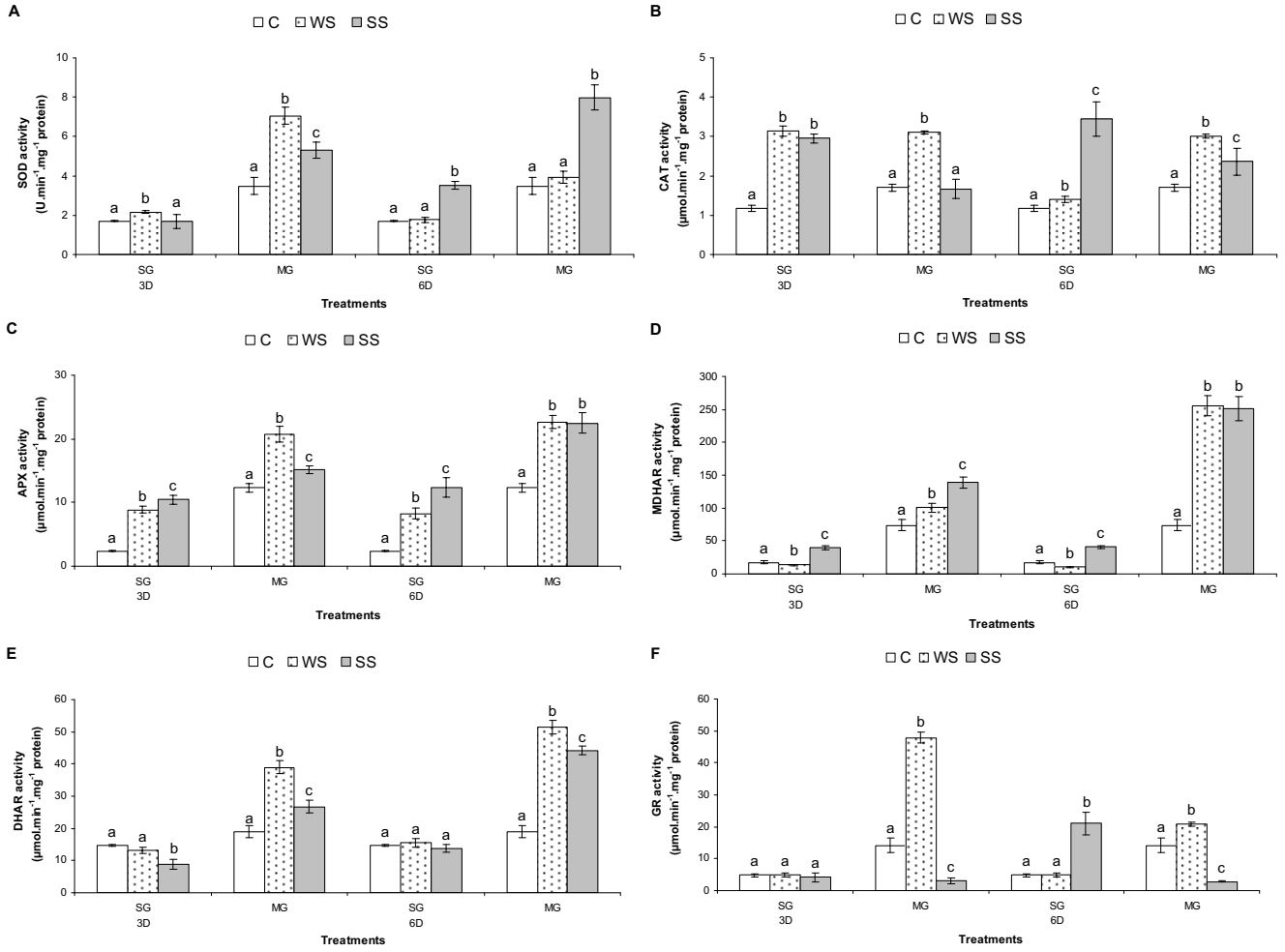

Fig. 1 Activity of superoxide dismutase (SOD; **A**) and catalase (CAT; **B**), ascorbate peroxidase (APX; **C**), monodehydroascorbate reductase (MDHAR; **D**), dehydroascorbate reductase (DHAR; **E**) and glutathione reductase (GR; **F**) in fruits of control plants (C) and stressed plants (S) of plants subjected to water stress (WS) or salt stresses (SS) for three and six days (3D and 6D). Values are the mean (± S.E.) of five replicates and different letters indicate significant differences determined by the Fisher test (LSD) ($P<0.05$). SG: Fruits at the small green stage, MG: Fruits at the mature green stage.

malized to transcript levels of *SlActin*. Data were expressed as relative values with respect to the value found in the control fruits and presented in **Table 5**.

Water stress treatments induced the expression of *SlAPXcyto* and *SlDHAR1* transcripts in SG and MG fruits but more slightly in MG fruits than in SG fruits. The *SlAPXt*, *SlMDHAR* and *SlGRcp* transcript levels increased in MG fruits while it decreased in SG fruits of plants subjected to water stress. *SlGRcyto* transcript level enhanced in SG fruits of plants stressed for 6 days and in MG fruits of plants stressed for 3 and 6 days. Moreover, we found an increase in transcript levels of all studied genes in fruits at MG between fruits of plants stressed for 3 days and fruits of plants stressed for 6 days (**Table 5**).

Concerning salt stress, in SG fruits of plants stressed for 3 days, *SlAPXcyto* and *SlDHAR1* transcript levels decreased, while *SlGRcyto* and *SlGRcp* transcript levels increased. In SG fruits of plants stressed for 6 days, the transcript levels of all studied genes decreased except *SlMDHAR* transcript level which was not affected. In MG fruits, all checked transcript levels increased in fruits of plants stressed for 3 days; however, they decreased in fruits of plants stressed for

Table 5 Relative transcript level of cytosolic APX (*SlAPXcyto*) thylakoid-bound APX (*SlAPXt*), *SlMDHAR*, *SlDHAR1*, cytosolic GR (*SlGRcyto*) and chloroplastic GR (*SlGRcp*) in fruits of control plants (C) and in fruits of plants subjected to water or salt stresses (S) for three and six days (3D and 6D). Data were expressed as relative values with respect to the value for the control fruits. Values are the mean (± S.E.) of five replicates, and different letters within columns indicate significant differences from the control value determined by the Fisher test (LSD) ($P<0.05$). SG: Fruits at small green stage, MG: Fruits at mature green stage.

		3D			6D		
		C	**Water stress**	**Salt stress**	**C**	**Water stress**	**Salt stress**
SlAPXcyto	SG	1.00 ± 0.2 a	5.77 ± 0.7 b	0.53 ± 0.1 b	1.00 ± 0.2 a	7.13 ± 0.9 b	0.59 ± 0.1 b
	MG	1.00 ± 0.2 a	1.80 ± 0.2 b	3.68 ± 0.4 b	1.00 ± 0.2 a	4.43 ± 0.5 b	1.68 ± 0.2 b
SlAPXt	SG	1.00 ± 0.2 a	0.48 ± 0.1 b	1.26 ± 0.2 a	1.00 ± 0.2 a	0.36 ± 0.1 b	0.34 ± 0.0 b
	MG	1.00 ± 0.2 a	2.47 ± 0.3 b	5.38 ± 0.6 b	1.00 ± 0.2 a	4.61 ± 0.5 b	0.44 ± 0.1 b
SlMDHAR	SG	1.00 ± 0.2 a	0.29 ± 0.0 b	1.17 ± 0.2 a	1.00 ± 0.2 a	0.25 ± 0.0 b	1.20 ± 0.2 a
	MG	1.00 ± 0.2 a	2.58 ± 0.3 b	1.98 ± 0.3 b	1.00 ± 0.2 a	3.71 ± 0.4 b	0.42 ± 0.1 b
SlDHAR1	SG	1.00 ± 0.2 a	7.07 ± 0.8 b	0.15 ± 0.0 b	1.00 ± 0.2 a	6.73 ± 0.7 b	0.07 ± 0.0 b
	MG	1.00 ± 0.2 a	3.37 ± 0.4 b	1.74 ± 0.2 b	1.00 ± 0.2 a	4.12 ± 0.5 b	0.32 ± 0.0 b
SlGRcyto	SG	1.00 ± 0.2 a	0.72 ± 0.1 a	1.57 ± 0.2 b	1.00 ± 0.2 a	4.33 ± 0.5 b	0.67 ± 0.1 b
	MG	1.00 ± 0.2 a	3.78 ± 0.4 b	4.55 ± 0.6 b	1.00 ± 0.2 a	6.23 ± 0.8 b	0.80 ± 0.1 a
SlGRcp	SG	1.00 ± 0.2 a	0.67 ± 0.1 b	2.31 ± 0.3 b	1.00 ± 0.2 a	0.58 ± 0.1 b	0.57 ± 0.1 b
	MG	1.00 ± 0.2 a	4.32 ± 0.5 b	3.27 ± 0.4 b	1.00 ± 0.2 a	9.32 ± 1.1 b	0.61 ± 0.1 b

6 days except *SlAPXcyto* transcript which increased and *SlGRcyto* transcript, which did not change (**Table 5**).

DISCUSSION

Exposure of plants to abiotic stresses such as drought, high salinity, extreme light and temperature leads to major loss in crop productivity worldwide (Shao *et al*. 2007). Among the various abiotic stress factors, drought and salt stresses affect almost every aspect of the physiology and biochemistry of plants and these constraints reduce dramatically plant yield (Bohnert and Jensen 1996). The aim of this study was to compare the effects of salt stress and water deficit on oxidative parameters and antioxidant defence mechanisms in tomato fruits. In previous work, Murshed *et al*. (2008a) have showed that fruits submitted to water stress responded as different manner to oxidative stress depending of age of fruit, so to compare effects of drought and salt, we have chosen fruits at two different stages: small green (SG fruits) and mature green (MG fruits) corresponding to the medium and the end of fruit growth phase. Preliminarily study also shown that salt stress (100 mM NaCl) and water stress applied by holding off irrigation induced the same decrease in leaf water potential ($L\Psi_w$), and it shown also that tomato fruits are highly affected by these stresses when they were applied during fruit growth phase.

The fruit water parameters ($F\Psi_w$, $F\Psi_O$, $F\Psi_t$ and FWC) and the fruit oxidative parameters (H_2O_2 and MDA) were determined. The mechanisms that could confer protection to oxidative stress were also investigated. For this purpose, AsA and DHA concentrations, and the activities of antioxidant enzymes: SOD and CAT as well as the activities and relative transcript levels of the enzymes of ascorbate-glutathione cycle (APX, MDHAR, DHAR and GR) were analysed.

Effect of water deficit and salt stress on fruit water parameters ($F\Psi_w$, $F\Psi_o$, $F\Psi_t$ and FWC) was evaluated as indicator of stress intensity and also as adaptive response to stress (**Table 2**). Fruit water content (FWC) was not affected by water and salt stresses. After 3 days of stress, similar decline in $F\Psi_w$ was found in fruits of plants subjected to salt stress and fruits of plants subjected to water stress. The decrease in $F\Psi_o$ and $F\Psi_t$ was only found in fruits subjected to salt stress. After 6 days of stress, $F\Psi_w$, $F\Psi_o$ and $F\Psi_t$ decreased in all stressed fruits except in SG fruits subjected to water stress, where $F\Psi_o$ not changed, and in MG fruits subjected to salt stress, where $F\Psi_w$ not changed and $F\Psi_t$ increased. This fact can suggest a better osmotic adjustment to maintain cell turgor in salt-stressed fruits compared to the water-stressed fruits by a possible salt accumulation in cells. The salt-stressed SG fruits seem to be more sensitive to stress than the MG fruits, but the $F\Psi_t$ increased between days 3 and 6. Compatible solutes accumulation in the cytoplasm is considered as a mechanism to contribute stress tolerance (Hare *et al.* 1998).

The H_2O_2 content was noticeably higher in MG fruits than that in SG fruits; however, MDA content was similar in fruits at both development stages (**Table 3**). The level of AsA and the AsA redox state were higher in MG fruits than that in SG fruits; furthermore, the higher AsA concentration in MG fruits was associated with a lower DHA concentration (**Table 4**). Moreover, the activities of all studied antioxidants enzymes were higher in MG fruits than that in SG fruits (**Fig. 1**). These results clearly established that the oxidative parameters (MDA and H_2O_2 contents) and antioxidant systems, enzymatic (SOD, CAT, APX, MDHAR, DHAR and GR) and non-enzymatic (AsA and DHA), were noticeably changed in fruit between the both stages of development. These changes in antioxidant systems during tomato fruit development were reported in other studies (Jiménez *et al.* 2002; Andrews *et al.* 2004). H_2O_2 is involved in virtually all major areas of aerobic biochemistry and is produced in copious quantities by several enzyme systems, even under optimal conditions (Noctor and Foyer 1998). The excess production of ROS during stress results from the inhibition of photosynthetic activity due to imbalance between light capture and its utilization (Foyer and Noctor 2005). Our results showed an increase in the H_2O_2 contents in water-stressed fruits at both development stages but H_2O_2 content in SG fruits were more important than in MG fruits (**Table 3**). In response to salt stress, H_2O_2 content increased only in SG fruits, but it decreased in MG fruits (**Table 3**) suggesting the sensitivity to stress of this development stage. The decrease of H_2O_2 levels compared to the control fruits, observed in fruits at MG stage could be related to the lower formation of H_2O_2 in fruits in response to salt stress (**Table 3**) or by a more active antioxidant system to eliminate ROS. In response to salt stress, increased H_2O_2 levels have been reported in different plant species (Kukreja *et al.* 2005; Sairam *et al.* 2005). In tomato, Mittova *et al.* (2002) observed a significant increase in H_2O_2 under salt stress.

The peroxidation of membrane lipids is a reliable indication of uncontrolled ROS production and hence of oxidative stress (Noctor and Foyer 1998). It is well known that ROS-induced peroxidation of membrane lipids is a reflection of stress-induced damage at the cellular level (Jain *et al.* 2001). Therefore, the level of MDA, a decomposition product of polyunsaturated fatty acids produced during peroxidation of membrane lipids, is often used as an indicator of oxidative damage (Mittler 2002). The results obtained in this study show an increase in MDA content in SG fruits subjected to water and salt stresses for 3 days and in MG fruits subjected to salt stress for 3 and 6 days. This increase of MDA content suggests that antioxidant systems were not efficient enough to counter the oxidative damage caused by stress. These results agree with previous results obtained in fruits of tomato cultivar Raissa, where water stress caused a significant increase in H_2O_2 and MDA contents of fruits (Murshed *et al.* 2008a). In contrast, these results do not agree with these of previous studies where a lower lipid peroxidation resulted from elevated activities of antioxidants under salt stress was reported in rice (Dionisio-Sese and Tobita 1998), sugar-beet (Bor *et al.* 2003) and tomato (Shalata *et al.* 2001).

Since the difference of behaviour between fruits subjected to salt stress and water deficit could be resulted from their capability to eliminate ROS (Hernàndez *et al.* 2001; Bor *et al.* 2003), the ascorbate levels and activities of antioxidant enzymes have been determined. Plants utilize both enzymatic and non-enzymatic antioxidant defence systems against oxidative damage. Among the non-enzymatic antioxidants, ascorbate (AsA) is found to be one of the best characterized compounds, required for many key metabolic functions in plant cells (Foyer 1993). In addition to its ability to directly scavenge ROS, ascorbate together with APX, MDHAR, DHAR and GR participates in one of the major H_2O_2-scavenging path ways in plant cells i.e. the ascorbate-glutathione cycle which plays a key role in the antioxidant defence system (Noctor and Foyer 1998).

It is generally assumed that plant tolerance to environmental stresses is positively correlated with AsA content (Tambussi *et al.* 2000). In our conditions, water stress did not affect AsA concentration in fruits; however, water stress increased DHA concentration and by consequence decreased the ascorbate redox state in SG fruits of plants stressed for 6 days (**Table 4**). These results do not agree with our previous results, where water stress caused a significant increase in AsA concentration in tomato fruits (Murshed *et al.* 2008a). However, the conditions of culture, the tomato cultivar, the duration and application of treatment were different.

Under salt stress, AsA concentration increased in all fruits of plants stressed except in SG fruits stressed for 3 days. DHA concentration increased in SG fruits of plants stressed for 3 days but it decreased with an increase in the ascorbate redox state in SG fruits of plants stressed for 6 days. DHA concentration decreased also in MG fruits of plants stressed for 3 days with an increase in the ascorbate redox state in these fruits (**Table 4**). These results agree with these of previous studies where a significant increase

in ascorbate was found in some plants subjected to salinity condition (Shalata *et al.* 2001). The AsA pool is generally determined by its rates of not only synthesis but also regeneration (Song *et al.* 2005). Therefore, the higher AsA concentration in fruits with a lower DHA concentration under salt stress treatments that we observed in our study could be explained by the increase of the stimulation of AsA synthesis (in MG salt stressed fruits for 6 days) and/or the AsA regeneration from MDHA and DHA.

The regulation of the activities of antioxidant enzymes is a rapid and efficient response to limit the excess of ROS generated by environmental stresses and it was observed in different fruits (Sofo *et al.* 2004). The antioxidant enzymes play different and complementary roles in the concerted cell defence, such as direct scavenging of ROS (Palatnik *et al.* 1999). In plants, this enzymatic response could constitute an adaptive advantage in the protection from oxidative stress. In our study, changes in the activities of antioxidant enzymes such as SOD and CAT as well as the ascorbate-glutathione cycle enzymes: APX, MDHAR, DHAR and GR were analysed under water and salt stresses (**Fig. 1**). Recycling enzymes are present in the diverse cell compartments such as chloroplast, mitochondria, cytosol. Because of determination of enzymatic activity of the several isoforms is not easy, the relative transcript levels of some genes encoding these enzymes: cytosolic *APX* (*SlAPXcyto*), thylakoid-bound *APX* (*SlAPXt*), *SlDHAR1*, *SlMDHAR*, cytosolic *GR* (*SlGRcyto*) and chloroplastic *GR* (*SlGRcp*) were also analysed under water and salt stresses (**Table 5**).

The $O_2^{\cdot-}$ radical is the main source of oxidative injury in plants and the dismutation of $O_2^{\cdot-}$ into H_2O_2 reaction catalyzed by the superoxide dismutase (SOD) maintains intracellular $O_2^{\cdot-}$ within normal levels and various authors have implicated this enzyme in the protection of cells from oxidative stress (Smirnoff 1993). In fact, our results show that water stress induce an increase in SOD activity in fruits of plants stressed for 3 days, but this effect disappeared after 6 days of treatment (**Fig. 1A**) while H_2O_2 levels continued to be elevated. Under salt stress, SOD activity increased in MG fruits of plants stressed for 3 days and in SG and MG fruits of plants stressed for 6 days (**Fig. 1A**). The increase of SOD activity in stressed plants may be interpreted as a direct response to augmented $O_2^{\cdot-}$ generation. This increase could enhance the ability of the fruits to scavenge $O_2^{\cdot-}$ radicals, which might lessen membrane damage. SOD induction by different environmental stresses has been previously reported in tomato leaves (Noctor *et al.* 2000; Mittova *et al*. 2004).

The H_2O_2 produced by SOD is itself a potent oxidizing agent. Therefore, it is essential for plants to have an effective means of detoxifying H_2O_2, a task that can be accomplished by CAT and APX (Fornazier *et al.* 2002 Bor *et al*. 2003). Our results show that the activity of CAT increased under water and salt stresses except in MG fruits of plants subjected to salt stress for 3 days (**Fig. 1B**). The increase in CAT activity under stress conditions has been reported in different plants (Kerdnaimongkol *et al.* 1997; Mittova *et al*. 2004; Jaleel *et al*. 2007).

APX are a family of isozymes localized in all the four major cellular compartments: stromal, thylakoid-membrane-bound APX in chloroplasts (APXt), microbody (including glyoxysomes and peroxisomes) membrane-bound APX, cytosolic APX (APXcyto), and lastly a mitochondrial-membrane-bound form (Shigeoka *et al*. 2002). In our study, APX activity was increased by all water and salt stress treatments (**Fig. 1C**). Moreover, changes in *SlAPXcyto* and *SlAPXt* transcripts were found under water and salt stresses but these changes were not directly related to changes in APX activity under these stresses (**Table 5, Fig. 1C**), suggested a post-transcription regulation. The increase in APX activity suggests that this is an adaptation to remove the excess H_2O_2 generated under stress conditions in combination with catalase. Numerous studies of ROS-scavenging enzymes in plants have demonstrated that APX activity generally increases in response to environmental stresses (Lopez *et al.* 1996; Shigeoka *et al.* 2002; Mittova *et al*. 2004). A recent transcriptome-based analysis confirms the susceptibility of *APXcyto* to environmental factors (Kim *et al.* 2007). Moreover, *APXcyto* transcript in pea and spinach leaves significantly increased in response to abiotic stresses (Hernàndez *et al.* 2000; Yoshimura *et al.* 2000). *APXt* transcript level change also in response to environmental stress (Tanabe *et al.* 2007). Ishikawa and Shigeoka (2008) reported that the expression of chloroplastic *APX* is post-transcriptionally regulated by alternative splicing.

The regeneration of ascorbate is a process of great importance in the antioxidant response. The first enzyme implicate in AsA regeneration is MDHAR, one of the key enzymes in the ascorbate–glutathione cycle (Noctor and Foyer 1998). In this study, under water stress, MDHAR activity and *SlMDHAR* transcript increased only in fruits at MG (**Fig. 1D, Table 5**). The MDHAR activity increased in the salt-stressed MG fruits more than in salt-stressed SG fruits (**Fig. 1D**). Moreover, *SlMDHAR* transcript increased in fruits at MG in plants stressed for 3 days but it decreased in plants stressed for 6 days (**Table 5**). Theses results may suggest that MDHAR is important to recycling ascorbate in MG fruits were it is abundant. The lower activity observed in SG fruits can contribute to sensitivity of SG fruits. Also the correlation that we found between MDHAR activity and *SlMDHAR* transcript under water stress agree with those of Jiménez *et al.* (2002) who reported that *MDHAR* transcript correlated well with MDHAR activity. However, in our study we did not find a good correlation between MDHAR activity and *SlMDHAR* transcript accumulation under salt stress, suggesting a post-transcription regulation.

DHAR, with the participation of reduced glutathione (GSH), catalyzes the reduction of DHA to AsA and thus contributes to the regulation of ascorbate redox state (Foyer and Noctor 2003). The increase in DHAR activity ensures efficient regeneration of AsA, which can scavenge higher levels of H_2O_2 under stress conditions. In the current study, in response to water stress, the increase in DHAR activity was only found in MG fruits (**Fig. 1E**). In contrast, the increase in *SlDHAR1* transcript was found in all fruits subjected to water stress (**Table 5**). Under salt stress, an increase in DHAR activity was also found in MG fruits (**Fig. 1E**). Changes in DHAR activity in response to stress were not directly related to changes in *SlDHAR1* genes expression (**Fig. 1E, Table 5**), suggested a post-transcription regulation or the existence of other genes coding for DHAR. The increase in DHAR activity in response to various abiotic stresses was reported in several studies (Ushimaru *et al.* 1992; Urano *et al.* 2000), suggesting that DHAR is important for maintenance of the cellular ascorbate pool and protection against such stresses.

Oxidized glutathione (GSSG) produced by DHAR activity is reduced to GSH by GR to allow the chain reactions of scavenging H_2O_2 by APX to be completed and continued (Mittler 2002; Apel and Hirt 2004). GR is present in several isoforms located in different cell compartments. Over-expression of GR increases antioxidant activity and improves tolerance to oxidative stress (Potters *et al.* 2004). Results obtained in this investigation reveal that in response to water stress, GR activity increased only in MG fruits, where *SlGRcyto* and *SlGRcp* transcripts increased also (**Fig. 1F, Table 5**). In fruits at SG, *SlGRcyto* transcript increased only in fruits of plants stressed for 6 days, while *SlGRcp* transcript decreased in fruits of plants stressed for 3 and 6 days (**Table 5**). Under salt stress, GR activity increased in SG fruits of plants stressed for 6 days, but it was decreased in MG fruits by all salt stress treatments (**Fig. 1F**). *SlGRcyto* and *SlGRcp* transcripts increased in SG and MG fruits of plants stressed for 3 days. In contrast, *SlGRcyto* decreased in SG fruits of plants stressed for 6 days, where *SlGRcp* transcript decreased also in fruits at both development stages (**Table 5**). These results show no correlation between GR activity and *SlGRcyto* and *SlGRcp* transcripts (**Fig. 1F, Table 5**), suggesting a post-transcription regulation.

CONCLUSION

From these results, it can be concluded that water and salt stress lead to oxidative stress and modulates the antioxidative responses of tomato fruits. The behaviour of the tomato plant under saline conditions appears to be not similar to that under drought conditions. In response to salt stress, tomato seems to maintain cell turgor while is more difficult during water stress. A perusal of the results shows that changes in the oxidative parameters, the ascorbate pool and the antioxidant enzymes were different under water stress from that under salt stress. Our study has also established the link between the regulation of antioxidant enzymes and ascorbate–glutathione cycle under both stresses and the fruit development stages. The results show that the AsA recycling enzymes (MDHAR, DHAR and GR) was induced by water deficit only in MG fruits. The same observations were noted for salt stress except for GR activity. This work underlines the importance of antioxidant enzymes and compounds in protecting cellular apparatus during stress conditions especially at mature green stage. The sensitivity of small green fruits could be explained by the minor activity of antioxidant defence system. On the other hand, post regulation expression of ascorbate recycling enzymes may be investigated during abiotic stress.

REFERENCES

Aebi H (1984) Isolation, purification, characterization, and assay of antioxygenic enzymes: catalase *in vitro*. *Methods in Enzymology* **105**, 121-126

Andrews PK, Fahy DA, Foyer CH (2004) Relationships between fruit exocarp antioxidants in the tomato (*Lycopersicon esculentum*) high *pigment-1* mutant during development. *Physiologia Plantarum* **120**, 519-528

Apel K, Hirt H (2004) Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. *Annual Review of Plant Biology* **55**, 373-399

Arora A, Sairam RK, Srivastava GC (2002) Oxidative stress and antioxidative systems in plants. *Current Science* **82**, 1227-1238

Asada K (1999) The water–water cycle in chloroplasts: scavenging of active oxygen and dissipation of excess photons. *Annual Review of Plant Physiology and Plant Molecular Biology* **50**, 601-609

Bohnert HJ, Jensen RG (1996) Metabolic engineering for increased salt tolerance: the next step. *Australian Journal of Plant Physiology* **23**, 661-667

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 Science* **164**, 77-84

Bowler C, Van Montagu M, Inzé D (1992) Superoxide dismutase and stress tolerance. *Annual Review of Plant Physiology and Plant Molecular Biology* **43**, 83-116

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

Dhindsa RS, Plumb-Dhindsa P, Thorpe TA (1981) Leaf senescent: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *Journal of Experimental Botany* **32**, 93-101

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

Dodds GT, Trenholm L, Rajabipour A, Madramootoo CA, Norris ER (1997) Yield and quality of tomato fruit under water-table management. *Journal of American Society for Horticultural Science* **122**, 491-498

Food and Agriculture Organization of the United Nations (FAO) Available online: http://www.fao.org

Fornazier RF, Ferreira RR, Pereira GJG, Molina SMG, Smith RJ, Lea PJ, Azevedo RA (2002) Cadmium stress in sugarcane in callus culture effects on antioxidant enzymes. *Plant Cell, Tissue and Organ Culture* **71**, 121-131

Foyer CH (1993) Ascorbic acid. In: Alscher RG, Hess JL (Eds) *Antioxidants in Higher Plants*, CRC Press Inc., Florida, pp 31-58

Foyer CH, Lelandais M, Kunert KJ (1994) Photooxidative stress in plants. *Physiologia Plantarum* **92**, 696-717

Foyer CH, Noctor G (2003) Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. *Physiologia Plantarum* **119**, 355-364

Foyer CH, Noctor G (2005) Oxidant and antioxidant signalling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. *Plant, Cell and Environment* **28**, 1056-1071

Grover A, Pareek A, Singla SL, Minhas D, Katiyar S, Ghawana S, Dubey H, Agarwal M, Rao GU, Rathee J, Grover A (1998) Engineering crops for tolerance against abiotic stresses through gene manipulation. *Current Science* **75**, 689-696

Hare PD, Cress WA, van Staden J (1998) Dissecting the roles of osmolyte accumulation during stress. *Plant, Cell and Environment* **21**, 535-553

Hernàndez JA, Ferrer MA, Jiménez A, Ros-Barcelo A, Sevilla F (2001) Antioxidant system and $O2^{\cdot-}/H_2O_2$ production in the apoplast of *Pisum sativum* L. leaves: its relation with NaCl-induced necrotic lesions in minor veins. *Plant Physiology* **127**, 817-831

Hernàndez JA, Jiménez A, Mullineaux PM, Sevilla F (2000) Tolerance of pea (*Pisum sativum* L.) to long-term salt stress is associated with induction of antioxidant defenses. *Plant, Cell and Environment* **23**, 853-862

Ishikawa T, Shigeoka S (2008) Recent advances in ascorbate biosynthesis and the physiological significance of ascorbate peroxidase in photosynthesizing organisms. *Bioscience, Biotechnology, and Biochemistry* **72**, 1143-1154

Jain M, Mathur G, Koul S, Sarin NB (2001) Ameliorative effects of proline on salt stressed lipid peroxidation in cell lines of groundnut (*Arachis hypogea* L.). *Plant Cell Reports* **20**, 463-468

Jaleel CA, Gopi R, Manivannan P, Panneerselvam R (2007) Responses of antioxidant defense system of *Catharanthus roseus* (L.) G. Don. to paclobutrazol treatment under salinity. *Acta Physiologiae Plantarum* **29**, 205-209

Jiménez A, Creissen G, Kular B, Firmin J, Robinson S, Verhoeten M, Mullineaux P (2002) Changes in oxidative processes and components of the antioxidant system during tomato fruit ripening. *Planta* **214**, 751-758

Kampfenkel K, Van Montagu M, Inze D (1995) Extraction and determination of ascorbate and dehydroascorbate from plant tissue. *Analytical Biochemistry* **225**, 165-167

Kerdnaimongkol K, Bhatia A, Jaly RJ, Woodson WR (1997) Oxidative stress and diurnal variation in chilling sensitivity of tomato seedlings. *American Society for Horticultural Science* **122**, 485-490

Kim DW, Shibato J, Agrawal GK, Fujihara S, Iwahashi H, Kim DH, Shim IS, Rakwal R (2007) Gene transcription in the leaves of rice undergoing salt-induced morphological changes (*Oryza sativa* L.). *Molecules and Cells* **24**, 45-59

Kukreja S, Nandwal AS, Kumar N, Sharma SK, Sharma SK, Unvi V, Sharma PK (2005) Plant water status, H_2O_2, scavenging enzymes, ethylene evaluation and membrane integrity of *Cicer arietinum* roots as affected by salinity. *Biologia Plantarum* **49**, 305-308

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. *Physiolgia Plantarum* **97**, 13-20

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

Mittova V, Guy M, Tal M, Volokita M (2002) Response of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennellii* to salt-dependent oxidative stress: increased activities of antioxidant enzymes in root plastids. *Free Radical Research* **36**, 195-202

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*. *Journal of Experimental Botany* **55**, 1105-1113

Mittova V, Tal M, Volokita M, Guy M (2003) Up-regulation of the leaf mitochondrial and peroxisomal antioxidative systems in response to salt-induced oxidative stress in the wild salt-tolerant tomato species *Lycopersicon pennellii*. *Plant Cell and Environment* **26**, 845-56

Murshed R, Lopez-Lauri F, Keller C, Monnet F, Sallanon H (2008a) Acclimation to drought stress enhances oxidative stress tolerance in *Solanum lycopersicum* L. fruits. *Plant Stress* **2**, 145-151

Murshed R, Lopez-Lauri F, Sallanon H (2008b) Microplate quantification of enzymes of the plant ascorbate–glutathione cycle. *Analytical Biochemistry* **383**, 320-322

Noctor G, Foyer CH (1998) Ascorbate and glutathione: keeping active oxygen under control. *Annual Review of Plant Physiology and Plant Molecular Biology* **49**, 249-79

Noctor G, Jovanovic SV, Foyer CH (2000) Peroxide processing in photosynthesis: antioxidant coupling and redox signalling. *Philosophical Transactions Royal Society London Ser.* **355B**, 1465-1475

Palatnik JF, Carrillo N, Valle EM (1999) The role of photosynthetic electron transport in the oxidative degradation of chloroplastic glutamine synthetase. *Plant Physiology* **121**, 471-478

Passam HC, Karapanos IC (2008) Eggplants, peppers and tomatoes: factors affecting the quality and storage life of fresh and fresh-cut (minimally processed) produce. In: Passam H (Ed) *The Fruiting Species of the Solanaceae*. *The European Journal of Plant Science and Biotechnology* **2 (Special Issue 1)**, 156-170

Potters G, Horemans N, Bellone S, Caubergs RJ, Trost P, Guisez Y, Asard H (2004) Dehydroascorbate influences the plant cell cycle through a glutathione-independent reduction mechanism. *Plant Physiology* **134**, 1479-87

Purvis AC, Shewfelt RL, Gegogeine JW (1995) Superoxide production by mitochondria isolated from green bell pepper fruit. *Plant Physiology* **94**, 743-749

Rengasamy P (2006) World salinization with emphasis on Australia. *Journal of Experimental Botany* **57**, 1017-1023

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 Science* **162**, 897-904

Sairam RK, Srivastava GC, Agarwal S, Meena RC (2005) Differences in

antioxidant activity in response to salinity stress in tolerant and susceptible wheat genotypes. *Biologia Plantarum* **49**, 85-89

Sairam RK, Tyagi A (2004) Physiology and molecular biology of salinity stress tolerance in plants. *Current Science* **86**, 407-421

Shalata A, Mittova V, Volokita M, Guy M, Tal M (2001) Response of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennellii* to salt-dependent oxidative stress: the root antioxidative system. *Physiologia Plantarum* **112**, 487-494

Shao HB, Chu LY, Wu G, Zhang JH, Lu, ZH, Hu YC (2007) Changes of some anti-oxidative physiological indices under soil water deficits among 10 wheat (*Triticum aestivum* L.) genotypes at tillering stage. *Colloids Surf B: Biointerfaces* **54**, 143-149

Shigeoka S, Ishikawa T, Tamoi M, Miyagawa Y, Takeda T, Yabuta Y, Yoshimura K (2002) Regulation and function of ascorbate peroxidase isoenzymes. *Journal of Experimental Botany* **53**, 1305-1319

Smirnoff N (1993) The role of active oxygen in the response to water deficit and desiccation. *New Phytologist* **125**, 27-58

Smirnoff N (1995) Antioxidant systems and plant response to the environment. In: Smirnoff N (Ed) *Environment and Plant Metabolism*, Bios Scientific Publishers, Oxford, UK, pp 217-243

Smirnoff N, Wheeler GL (2000) Ascorbic acid in plants: biosynthesis and function. *Critical Reviews in Plant Sciences* **19**, 267-290

Sofo A, Dichio B, Xiloyannis C, Masia A (2004) Effects of different irradiance levels on some antioxidant enzymes and on malondialdehyde content during rewatering in olive tree. *Plant Science* **166**, 293-302

Song XS, Hu WH, Mao WH, Ogweno JO, Zhou YH, Yu JQ (2005) Response of ascorbate peroxidase isoenzymes and ascorbate regeneration system to abiotic stresses in *Cucumis sativus* L. *Plant Physiology and Biochemistry* **43**, 1082-1088

Sonnefeld C, Van der Wees A (1984) Voedingsoplossing voor de teelt van tomaten in steenwol. Proefstation voor de Tuinbouw onder glas-Consulentshap voor de Tuinbouw te Naaldwijk. Informatiereeks, Naaldwijk, Netherlands, 63 pp

Tambussi EA, Bartoli CG, Beltrano J, Guiamet JJ, Araus JL (2000) Oxidative damage to thylakoid proteins in water-stressed leaves of wheat (*Triticum aestivum*). *Physiologia Plantarum* **108**, 398-404

Tanabe N, Yoshimura K, Kimura A, Yabuta Y, Shigeoka S (2007) Differential expression of alternatively spliced mRNAs of *Arabidopsis* SR protein homologs, atSR30 and atSR45a, in response to environmental stress. *Plant and Cell Physiology* **48**, 1036-1049

Urano J, Nakagawa T, Maki Y, Masumura T, Tanaka K, Murata N, Ushimaru T (2000) Molecular cloning and characterization of a rice dehydroascorbate reductase. *FEBS Letters* **466**, 107-111

Ushimaru T, Shibasaka M, Tsuji H (1992) Depelopment of the $O_2^{\cdot-}$ detoxification system during adaptation to air of submerged rice seedlings. *Plant and Cell Physiology* **33**, 1065-1071

Verma S, Mishra SN (2005) Putrescine alleviation of growth in salt stressed *Brassica juncea* by inducing antioxidative defense system. *Journal of Plant Physiology* **162**, 669-677

Wild A (2003) *Soils, Land and Food: Managing the Land during the Twenty-First Century*, Cambridge University Press, Cambridge

Willekens H, Chamnongpol S, Davey M, Schraudner M, Langebartels C, Van Montagu M (1997) Catalase is a sink for H_2O_2 and is indispensable for stress defence in C3 plants. *The EMBO Journal* **16**, 4806-4816

Yoshimura K, Yabuta Y, Ishikawa T, Shigeoka S (2000) Expression of spinach ascorbate peroxidase isoenzymes in response to oxidative stresses. *Plant Physiology* **123**, 223-234

Zhu Z, Wei G, Li J, Qian Q, Yu J (2004) Silicon alleviates salt stress and increases antioxidant enzymes activity in leaves of salt-stressed cucumber (*Cucumis sativus* L.). *Plant Science* **167**, 527-533

Zou L, Li H, Ouyang B, Zhang J, Ye Z (2006) Cloning and mapping of genes involved in tomato ascorbic acid biosynthesis and metabolism. *Plant Science* **170**, 120-127