Comparison of an antioxidant system in tolerant and susceptible wheat seedlings in response to salt stress

Koauthar Feki1,2, Sana Tounsi2 and Faıçal Brini1

1University of Carthage–Tunis, Centre of Biotechnology: Bordj Cedria, Laboratory of Legumes, BP 901, 2050 Hammam Lif, Tunisia. 2University of Sfax, Centre of Biotechnology of Sfax, Biotechnology and Plant Improvement Laboratory, B.P 1177, 3018 Sfax, Tunisia.

Abstract

It has been demonstrated previously that the physiological and molecular analysis of seedlings of the tolerant (Om Rabia3) and susceptible (Mahmoudi) Tunisian wheat genotypes were different at short and long-term response to salinity. In this study, we examined the antioxidant defence system in seedlings of these two cultivars at short-term response to different NaCl concentrations. The findings showed that high salinity tolerance of cv. Om Rabia3, as manifested by lower decrease in its dry biomass, was associated with lower malondialdehyde and hydrogen peroxide contents, lower accumulation of the superoxide (O₂⁻) in the roots and the shoots, and also lower decrease in ascorbate content than those in cv. Mahmoudi. Moreover, the expression of some genes coding for antioxidant enzymes such as the catalase, the superoxide dismutase and the peroxidase were enhanced by NaCl stress especially in the salt-tolerant cultivar. In parallel, their activities were increased in response to the same condition of stress and especially in the cv. Om Rabia3. Taken together, these data suggested that the capacity to limit oxidative damage is important for NaCl tolerance of durum wheat.

Additional keywords: antioxidant; oxidant; reactive oxygen species; salinity; Triticum durum.

Abbreviations used: APX (ascorbate peroxidase); AsA (ascorbic acid); CAT (catalase); DW (dry weight); GPX (glutathione peroxidase); GR (glutathione reductase); GSH (glutathione); MDA (malondialdehyde); NBT (nitroblue tetrazolium); POD (peroxidase); PMSF (phenylmethylsulfonyl fluoride); ROS (reactive oxygen species) SOD (superoxide dismutase); TCA (trichloroacetic acid).

Authors' contributions: Conceived and designed the experiments, statistical analysis and data interpretation: KF and FB. Performed the laboratory experiments: KF and ST. Wrote the paper: KF. Supervised and coordinated the work: FB.

Citation: Feki, K.; Tounsi, S.; Brini, F. (2017). Comparison of an antioxidant system in tolerant and susceptible wheat seedlings in response to salt stress. Spanish Journal of Agricultural Research, Volume 15, Issue 4, e0805. https://doi.org/10.5424/sjar/2017154-11507

Received: 06 Apr 2017. Accepted: 22 Nov 2017.

Copyright © 2017 INIA. This is an open access article distributed under the terms of the Creative Commons Attribution (CC-by) Spain 3.0 License.

Funding: Ministry of Higher Education and Scientific Research, Tunisia.

Competing interests: The authors have declared that no competing interests exist.

Correspondence should be addressed to Faiçal Brini: faical.brini@cbs.rnrt.tn

Introduction

In the developing and under developed world, about 2.5 billion people survive on wheat as an element source of energy (http://faostat.fao.org). In many arid and semi-arid regions in the world, wheat production yield is limited by the availability of water resources, the use of poor water quality and also when soil drainage is poor. Salinity is one of the major abiotic stress issues worldwide affecting plant cultivation and productivity, and its adverse impacts are getting more serious problem in regions where saline water is used for irrigation (Türkan & Demiral, 2009). The sodium exclusion from the leaves is the major mechanism that confers salt tolerance in wheat (Gorham et al., 1990; Husain et al., 2003). In general, the hexaploid bread wheat (Triticum aestivum: AABBDD) has a better capacity to exclude Na than durum wheat (Triticum durum: AABBB), which is linked to the Knal locus on chromosome 4D (Dvorák et al., 1994). High salinity causes a primary effect like hyperosmotic stress and ion disequilibrium producing secondary effects, such as ion toxicity, oxidative stress, hormonal imbalances and nutrient disturbances (Ashraf, 2009; Türkan & Demiral, 2009). In plant cells, salinity induces the production of reactive oxygen species (ROS) such as superoxide radical (O₂⁻), hydrogen peroxide (H₂O₂), and hydroxyl radical (OH). ROS are continuously generated during normal metabolic processes in the mitochondria, peroxisomes and cytoplasm. But, at high level they are highly cytotoxic and can react with vital biomolecules and consequently causing peroxidation, protein denaturation and DNA mutation (Choudhury et al., 2013; Hossain et al., 2015).
In plant cells, a complex defense system is implicated to maintain the redox homeostasis, which comprise of enzymatic and non-enzymatic components. In plants, many genes encode for different ROS-detoxifying or ROS-producing enzymes forming well organized ROS gene web (Mittler et al., 2004). Several enzymes are involved in the detoxification of ROS like superoxide dismutase (SOD; EC 1.15.1.1), ascorbate peroxidase (APX; EC 1.11.1.11), catalase (CAT; EC 1.11.1.6), glutathione reductase (GR; EC 1.6.4.2) and glutathione peroxidase (GPX; EC 1.11.1.9). SOD is the first enzyme implicated in the removal of ROS, and it catalyzes the conversion of O$_2^-$ to H$_2$O$_2$, and it occurs ubiquitously in every plant cell (Breusegem et al., 1999; Ashraf, 2009). H$_2$O$_2$ is a toxic ROS and has deleterious effects in plant tissue. It is scavenged by CAT and different classes of peroxidase (POD) (Mhamdi et al., 2010). Other enzymes that are very important in the ROS scavenging system and function in the ascorbate-glutathione cycle are glutathione reductase (GR), monodehydroascorbate reductase (MDHAR; EC 1.6.5.4) and dehydroascorbate reductase (DHAR; EC 1.8.5.1) (Foyer & Noctor, 2011). Several genes encoding for plant antioxidant enzymes have been cloned, characterized, and used in the construction of transgenic lines. Transgenic plants overexpressing SOD (Bowler et al., 1991; Van Camp et al., 1996; Breusegem et al., 1999; Alscher et al., 2002; Feki et al., 2016), APX (Wang et al., 1999), GR (Foyer et al., 1995), GPX (Roxas et al., 1997, 2000) and CAT (Feki et al., 2015) showed tolerance to various abiotic stresses.

It has been demonstrated that NaCl tolerance is closely correlated with the antioxidant capacity in numerous plants, such as bread wheat (Sairam et al., 2002, 2005; Mandhania et al., 2006; Choudhury et al., 2013; Hossain et al., 2015). However, it has been showed that the increase of the activity of some antioxidant enzymes is concomitantly with salt sensitivity. For example, in the leaves of the salt-sensitive rice plants, NaCl stress preferentially enhanced the activities of some antioxidant enzymes like SOD and APX (Lee et al., 2001). In addition, it has been reported that the responses of plant antioxidant enzymes to salinity show varying activity patterns according to the species and analyzed tissues (Gossett et al., 1994).

The non-enzymatic antioxidant system includes ascorbic acid (AsA), glutathione (GSH), α-tocopherols (vitamin E), flavonoids, anthocyanins, polyphenolic compounds and carotenoids (Schafer et al., 2002; Suzuki et al., 2012). The most abundant soluble antioxidants in plants are AsA and GSH, which play a key role in plant defense against oxidative stress (Foyer & Noctor, 2011; Venkatesh & Park, 2014). α-tocopherol, an abundant vitamin E compound, is a lipid soluble antioxidant found in chloroplasts where it counteracts lipid peroxidation through scavenging of lipid peroxyl radicals and detoxifies singlet oxygen and hydroxyl radicals (Munné-Bosch, 2005). Oxidative stress activates the expression of genes responsible for the synthesis of tocopherols in higher plants, generating its accumulation in plants (Shao et al., 2007; Wu et al., 2007).

In a previous work, the physiological analysis of the two Tunisian durum wheat genotypes showed differential tolerance to salinity (Brini et al., 2009). However, data concerning the effects of salinity on ROS production and antioxidant defense system in these two durum wheat cultivars are still lacking. In this work, we aimed to provide an insight view to antioxidant enzyme system in two Tunisian durum wheat treated with different NaCl stress conditions, in order to understand the mechanisms relevant in salt tolerance.

**Material and methods**

**Plant material, stress conditions and dry weight determination**

Two Tunisian cultivars of durum wheat (*Triticum turgidum* L. subsp. *durum*), Mahmoudi (salt sensitive) and Om Rabia3 (salt tolerant) were sterilized and then germinated on Petri dishes as described by Brini et al. (2009). Three-day-old seedlings were transferred to containers with modified half-strength Hoagland’s solution (Epstein, 1972). After 4 days, they were transferred to the same medium containing or not different NaCl concentrations (0, 50, 100 and 200 mM) and kept in stress for 3 days. All seedlings were grown in a glasshouse at 25 ± 5°C, 16 h photoperiod and 60 ± 10% relative humidity. After three days of NaCl exposure, five plants per each salt treatment were used for the determination of the dry weight (DW), malonyldialdehyde (MDA) and H$_2$O$_2$ contents, the antioxidative enzyme assays, and ascorbate content. All these experiments were repeated at least five times.

To determine the DW, shoots (leaf and sheath) were separated from roots, dried at 70°C and then weighed. Besides, fresh shoots samples from each plant were immediately frozen in liquid nitrogen and stored at 80°C, until performing the biochemical analysis.

**Lipid peroxidation**

The extent of lipid peroxidation was estimated by determining the amount of MDA in the leaves by the method of Ben Amor et al. (2005). Fresh shoots (0.15 g) were homogenized in 1.5 mL of 0.1% (w/v)
trichloroacetic acid (TCA) solution. The homogenate was centrifuged at 15,000 × g for 30 min. To 0.5 mL aliquot of the supernatant, 1.0 mL of 0.5% (w/v) thiobarbituric acid (TBA) in 20% (w/v) TCA solution was added. The mixture was heated at 90°C for 30 min, and then cooled on ice. The MDA equivalent was calculated by measuring absorbance at 532 and 600 nm in reference to a MDA standard curve.

**Quantitative H$_2$O$_2$ measurement**

The concentration of H$_2$O$_2$ was appraised following Velikova et al. (2000). Fresh shoots tissue (0.5 g) was homogenized with 5 mL of 0.1% (w/v) TCA. This homogenate was then centrifuged at 12,000 × g for 15 min. To 0.5 mL of the supernatant, 10 mM phosphate buffer (pH 7.0) and 1 M potassium iodide were added. The mixture was then vortexed and its absorbance was read at 390 nm, and H$_2$O$_2$ content was calculated using a standard curve with concentration ranging from 0.05 to 0.1 mM.

**Superoxide radical staining assay**

In order to detect O$_2^-$, we performed a staining assay based on nitroblue tetrazolium (NBT) (Jabs et al., 1996), in different parts of the two Tunisian wheat cultivars treated or not with 200 mM NaCl for 3 days. The reduction of NBT to insoluble blue formazan was used as a probe for O$_2^-$ generation. The samples were placed in the NBT solution (0.1 mM NBT, 25 mM HEPES pH 7.6) and subjected to vacuum infiltration for 5 min. Then, the samples were incubated under dark conditions for 2 h. Finally, they were treated with 80% ethanol and photographed. This staining assay was repeated three times using three to six different plants from each cultivar.

**Ascorbate content**

Ascorbate (AsA) was estimated following the method described by Mukherjee & Choudhuri (1983). Shoots material (0.25 g) was extracted with 10 mL of 6% (w/v) TCA solution. An aliquot of the extract was mixed with 2% dinitrophenyl hydrazine (in acidic medium) followed by the addition of one drop of thio-urea (in 70% ethanol). The mixture was boiled for 15 min in a water bath and after cooling at room temperature, 5 mL of 80% (v/v) H$_2$SO$_4$ were added to the mixture at 0°C. The absorbance was read at 530 nm. The levels of pure AsA were calculated from a standard curve plotted with varying known concentrations of pure AsA (Sigma-Aldrich).

**RNA extraction and semi-quantitative RT-PCR**

Total RNA from shoots treated or not with NaCl (200 mM NaCl) for 3 days was extracted using the TRIZOL method (Invitrogen). To remove contaminating DNA, total RNA (10 µg) of each treatment was treated with RNase-free DNasel (Promega) at 37°C for 15 min and further incubated at 65°C for 10 min. Then, the cDNA was synthesized using 0.5 µg of DNase treated RNA samples, M-MLV reverse transcriptase (Invitrogen) and the oligo-dT (18 mer) primer. One microliter of each cDNA was used for PCR amplification with the corresponding primers and the wheat’s actin gene was used as an internal control for gene expression (Table 1). The products were separated by electrophoresis on 1.5% agarose gel and quantified using the Gel DocXR Gel Documentation System (Bio-Rad). This software was used to calculate average band density which was recorded and used in graphic analyses. Band density was determined by this software and was given in arbitrary units and graphed using Microsoft Excel. The error bar was determined from three separate biological replicates. Each of three biological replicates consisted of pooled plants subjected or not to different stress conditions.

**Protein extraction**

Aliquots of frozen fresh shoot material (0.5 g) were ground to a fine powder with liquid nitrogen and homogenized in 1.5 mL of a cold solution containing

---

**Table 1. Sequences of primers used for semi-quantitative RT-PCR analyses of CAT, MnSOD and APX genes and their accession numbers**

| Primer | Nucleotide sequence (5'-3') | Annealing site | Accession No. |
|--------|-----------------------------|----------------|---------------|
| ACTF   | GTGCCCAATTTCAGAAAGGATA      | Actin          | AY663392      |
| ACTR   | GAAGACTCTCCATCGCAGATCAT     |                |               |
| CATF   | CGACTTCGCCGCCGAGCCGACGAC    | CAT            | GU984379      |
| CATR   | GCCGGATCCATCTGGTTGATGATATC  |                |               |
| SODF   | GAAGACCACGCGCACTACGTGCGC    | MnSOD          | EF392662      |
| SODR   | TACACGCAAGCAGCTTTTCTACACTC  |                |               |
| APXF   | ATGGCGGCTCCGGTGTTGGAGCACC   | APX            | EF555121      |
| APXR   | TACAATATCTTTGCTCTTTGACC    |                |               |
100 mM Tris-HCl buffer (pH 8.0), 10 mM EDTA, 50 mM KCl, 20 mM MgCl₂, 0.5 mM PMSF, and 2% (w/v) PVP. The homogenate was centrifuged at 14,000 × g for 30 min at 4°C, and the supernatant was used for determination of the antioxidative enzyme activities. Protein concentration was determined according to Bradford (1976).

**Enzyme assays**

The activity of SOD was monitored by the inhibition of photochemical reduction of NBT at 560 nm. The activity of SOD was determined by adding 50 µL of the enzymatic extract to a solution containing 50 µM NBT, 1.3 µM riboflavin, 13 mM methionine, 75 mM EDTA and 50 mM phosphate buffer (pH 7.8) (Giannopolitis & Ries, 1977).

CAT activity was determined according to Aebi (1984), by monitoring the disappearance of H₂O₂. Crude enzyme extract (0.1 mL) was added to 3 mL reaction mixtures containing 50 mM phosphate buffer (pH 7), 30 mM H₂O₂. Changes in optical density (OD) of the reaction solution at 240 nm were recorded every 20 s.

The activity of POD was determined according to Maehly & Chance (1954) by the guaiacol oxidation method. The final volume (3 mL) of the reaction mixture for POD contained 0.1 mL enzyme extract as well as 50 mM phosphate buffer (pH 7), 20 mM guaiacol, and 40 mM H₂O₂. Changes in OD of the reacted samples at 470 nm were recorded every 20 s.

APX activity was appraised following the method of Nakano & Asada (1981) with minor modifications. An aliquot of 0.2 mL enzyme extract was added to a mixture containing 0.8 mL of 50 mM potassium phosphate buffer (pH 7), 0.5 mM AsA, and 0.1 mM H₂O₂. The decrease in absorbance at 290 nm was recorded for 1 min. The enzyme activity of SOD, CAT, POD and APX was expressed as units/mg protein. One unit of SOD was defined as the enzyme quantity required causing 50% inhibition of the rate of NBT reduction at 560 nm in comparison with tubes lacking the plant extract. One unit of CAT and APX was defined as µmol/mL H₂O₂ decomposed per minute. One enzyme unit of POD is defined as change in 1 unit of absorbance per minute.

**Electrophoresis and enzyme activity staining**

Native-PAGE was carried out using 10% resolving gel at 4°C. POD isoforms were visualized by incubating the gel for 30 min in a 0.1 M sodium acetate buffer (pH 4.0) containing 1% (v/v) guaiacol. Then, gel was placed in a solution containing 4.7 mM 3-amino-9-ethylicarbazole, 38 mM N,N-dimethyl formamide, 0.1 M sodium acetate buffer (pH 5.0), 0.1 M CaCl₂ and 30% (v/v) H₂O₂. POD isoforms were appeared with brown bands after 10 to 20 min at 4°C (Vallejos, 1983). This experiment was repeated three times with similar results.

**Statistics analysis**

Data were analyzed using one-way ANOVA implemented in the SPSS software 13. Treatment mean separations were performed using Duncan’s multiple range tests.

**Results**

**Effect of NaCl concentration on shoots and roots growth**

NaCl stress significantly reduced shoots and roots dry biomass of both durum wheat cultivars. However, this decrease was more pronounced in the salt-sensitive cultivar (Mahmoudi) than in the tolerant one (Om Rabia3). Indeed, the root growth of the sensitive cultivar was extensively inhibited at NaCl concentration up to 100 mM. In contrast, in the presence of low Na⁺ in the medium (50 mM NaCl), shoots growth of these two wheat cultivars decreased significantly (Fig. 1).

**Oxidative stress evaluation**

In the present study, MDA content in the shoots of the two Tunisian durum wheat cultivars correlated with growth inhibition produced by NaCl stress. Moreover, MDA content increased significantly in cv. Mahmoudi shoots as compared to the other cultivar with the increase of NaCl concentration in the medium (Fig. 2).

Under standard conditions, a weak NBT staining was observed in the different parts of these two wheat cultivars. After salt stress and in contrast to the cv. Om Rabia3, an intense NBT staining was observed on cv. Mahmoudi leaves and roots, indicating an increase in the amount of O₂⁻ (Fig. 3).

**Ascorbate accumulation**

In this study, shoots ascorbate content in both durum wheat cultivars was reduced by NaCl treatment.
However, this decrease was significant in cv. Mahmoudi as compared to that in cv. Om Rabia3. Highest NaCl concentration in the medium caused a maximal decrease in ascorbate content in cv. Mahmoudi as compared to the other salt levels. Nevertheless, concerning the cv. Om Rabia3 the ascorbate content was stable at this high NaCl concentration (Fig. 4).

Expression analysis of CAT, MnSOD and APX genes

To monitor the expression profile of the three genes CAT, MnSOD and APX under salt stress, we performed RT-PCR analysis on the shoots of these two cultivars treated or not with NaCl for 3 days. Basal expression levels of these three genes were detected in the leaves of the non treated plants. After NaCl treatment, a significant increase in the level of these genes, about two to four times, was observed in the case of these two wheat cultivars. However, in the cv. Om Rabia3 the transcript accumulation of these genes was higher than in the cv. Mahmoudi. In addition, CAT and MnSOD transcripts were significantly accumulated under NaCl treatment compared to APX transcript in the cv. Om Rabia3 (Fig. 5).

Correlation between salinity and the activity of some antioxidant enzymes

In the present study, we observed a differential response in SOD activity in the two Tunisian wheat cultivars. Indeed, SOD activity of the salt tolerant cultivar (Om Rabia3) increased markedly with the increase in the external NaCl concentration, to attain a final activity about 5 times relative to unstressed plants. By contrast, SOD activity in the cv. Mahmoudi increased slightly and reached only about 3 times relative to the control plants.

Like SOD activity, the increase of CAT activity in these cultivars was different, and the CAT activity of cv. Om Rabia3 was higher than that in the cv. Mahmoudi in the presence of the three NaCl concentrations (Fig. 6).

In the two wheat cultivars, POD and APX activities increased significantly under salt stress compared to the non-treated plants. Moreover, this increase was higher in cv. Om Rabia3 than in cv. Mahmoudi. In the presence of high NaCl concentration in the medium (200 mM NaCl), POD activity of the salt-tolerant cultivar was about the double relative to the control plants and higher than that in cv. Mahmoudi. However, APX activity decreased in these two cultivars, compared to the treatment with medium NaCl concentration (100 mM NaCl). In addition, this decrease was significant in...
the cv. Mahmoudi compared to the other cultivar (Fig. 6).

**Peroxidase isoform visualization**

The isoenzyme composition of POD in these durum wheat cultivars treated or not with 200 mM NaCl was studied using a native PAGE. In normal condition, three main POD isoforms were revealed in these two cultivars. In the presence of 200 mM NaCl, an enhanced intensity of POD 3 isoform occurred in the two cultivars. However and compared to cv. Mahmoudi, an intense staining of POD 2 isoform was detected in the case of cv. Om Rabia3 treated with NaCl treatment (Fig. 7).

**Discussion**

Salinization of cropland in the Mediterranean region is a major limitation to crop yields. The ability to limit the accumulation of Na⁺ in leaves may be an important mechanism in salt tolerance because the excessive accumulation of Na⁺ causes the premature senescence of leaves (Pardo, 2010). In a previous work, it has been demonstrated that the response to NaCl treatment is different between the two Tunisian wheat cultivars (Om Rabia3 and Mahmoudi) at short and long-term. In this study, these two Tunisian wheat genotypes were grown for seven days and then subjected to NaCl treatment for short period (3 days). Significant differences of shoots and roots DW were detected between the non-treated and the stressed plants. Moreover, the salt-tolerant cultivar (Om Rabia3) presented higher roots and shoots DW than the salt-sensitive cultivar (Mahmoudi) under high NaCl concentration (Fig. 1). Similar to our findings, dry weight was less affected in salt tolerant sesame, sugar beet and moderately salt tolerant cotton (Greenway & Munns, 1980; Koca et al., 2007).

Salt stress induces oxidative damage in many plants like pea, rice and tomato (Gómez et al., 1999; Hernández et al., 2001; Uchida et al., 2002; Mittova et al., 2003). The MDA content is considered as an indicator of the level of lipid peroxidation. Many studies...
Comparison of an antioxidant system in tolerant and susceptible wheat seedlings

Figure 5. (a) Expression analysis of the CAT, MnSOD and APX genes in the shoot of the two durum wheat cultivars treated (S) or not (C) with salt for 3 days. MH, Mahmoudi; OR3, Om Rabia3. Actin gene was used as the positive control; (-) negative control without cDNA. (b) The histograms correspond to the band densities in the gels which are expressed in arbitrary units calculated by the analysis Gel DocXR software. The standard error was determined from three independent biologic replicates.

Figure 6. Antioxidant enzyme responses to NaCl treatments in durum wheat cultivars (MH, Mahmoudi; OR3, Om Rabia3). Values are means of 5 replicates ± SD (n = 5). Values having the same letter in each NaCl concentration are not significantly different at $p < 0.05$. 
showed that abiotic stresses lead to lipid peroxidation and consequently produce an accumulation of the MDA (Chaoui et al., 1997; Mandhania et al., 2006; Distelbarth et al., 2012). In agreement with these observations, our results showed that salinity induced oxidative stress in these two Tunisian durum wheat leaves, manifested by an accumulation of $O_2^-$ and an increase in MDA and $H_2O_2$ contents (Figs. 2, 3). Nevertheless, this increase was different between these two cultivars. Indeed, under NaCl treatment the level of lipid peroxidation and $H_2O_2$ content were higher in the cv. Mahmoudi than in the other cultivar. These data suggest that the cv. Om Rabia3 was better protected against oxidative damage under NaCl stress. Parallel to our results, low MDA content is important in terms of salt tolerance as represented in different studies. Salt tolerant barley (Liang et al., 2003), tobacco (Ruiz et al., 2005) and wheat cultivars (Sairam et al., 2002, 2005; Mandhania et al., 2006) also showed low level of lipid peroxidation which is important sign of higher oxidative damage limiting capacity under salinity.

AsA is the major antioxidant in the plant cell. As the generation of ROS is increased under stress conditions, AsA is believed to contribute actively in enhancing tolerance to various environmental stresses (Noctor & Foyer, 1998). It is clear that high level of endogenous AsA is essential effectively to maintain the antioxidant system that protects plants from oxidative damage due to abiotic and biotic stresses (Shigeoka et al., 2002). Salt induces a decrease in AsA content in wheat at the vegetative stage (Sairam et al., 2005) and at the reproductive stage (Athar et al., 2008). In agreement with this observation, our study showed that salinity induces a decrease in AsA in the two durum wheat cultivars particularly in the cv. Mahmoudi (Fig. 4). This larger AsA decrease was also found in salt sensitive pea cultivar (Hernández et al., 2001).

In Arabidopsis cells exposed to oxidative stress, some gene showed changes in expression levels. These genes encoded for proteins with antioxidant functions or were associated with defense responses or other stresses (Desikan et al., 2001). For example, there are many reports on the changes in the activity of various SOD isoenzymes and corresponding mRNA under osmotic stresses (Zhu & Scandalios, 1994). Our results showed that higher NaCl concentration produces an elevate expression of CAT, MnSOD and APX genes (Fig. 5). Interestingly, the higher expression of these three genes was observed in the cv. Om Rabia3. APX gene is induced by various stress conditions suggesting his crucial role in many stress tolerance like drought and heat shock (Mittler & Zilinskas, 1992). Concerning the antioxidative enzymes activities, NaCl stress caused up-regulation of the activities in these wheat cultivars. Comparing the enzymatic activity in the two Tunisian durum wheat cultivars, the higher activities were obtained in the salt tolerant cultivar (Om Rabia3) than in the sensitive one (cv. Mahmoudi). This result suggested that the cv. Om Rabia3 presents higher capacity for scavenging ROS than the cv. Mahmoudi. Thus, the decrease in the content of $H_2O_2$ in cv. Om Rabia3 is the result of SOD reaction which is accompanied by an increased enzymatic capacity to decompose it. This was particularly clear in cv. Om Rabia3, in which a greater and parallel increase in SOD, CAT, POD, and APX activities occurred under low or high salinity (Fig. 6). The correlation between salinity tolerance and the increase of SOD activity has been demonstrated in many works (Hernández et al., 2001; Sreenivasulu et al., 2000; Ben Amor et al., 2005; Mandhania et al., 2006; Distelbarth et al., 2011). Increased SOD activity in the cv. Om Rabia3 at higher NaCl level probably coped with injuring effects of $O_2^-$ . This suggestion was confirmed by the low amount of $O_2^-$ in the different part of this cultivar. APX utilizes AsA as its specific electron donor to reduce $H_2O_2$ to water with the generation of monodehydroascorbate (Shigeoka et al., 2002), and plays a crucial role in the management of ROS during stress (Ahmad et al., 2008). It has been reported previously that salinity increases APX activity in many salt tolerant cultivars (Hernández et al., 2001; Sairam et al., 2005; Koca et al., 2007). The susceptibility of the cv. Mahmoudi to salinity could be due to the inhibition of APX activity at higher salinity level. This inhibition was also observed previously in two bread wheat cultivars HD 2009 and HD 2687 (Sairam et al., 2005). Concerning POD, our results showed that salt stress produces an enhancement of POD activity in the two durum wheat cultivars. Nevertheless, POD activity remained unchanged in the salt sensitive cv.
Mahmoudi treated with high NaCl concentration. Interestingly, the increase of POD activity in cv. Om Rabia3 is concomitant with the enhanced intensity of POD2 isoform, which probably produces more activity in this cultivar than in the cv. Mahmoudi. CAT is the main scavenger of strong oxidant H$_2$O$_2$ in peroxisomes and it converts H$_2$O$_2$ to water and molecular oxygen (Willekens et al., 1995). Higher activities of CAT and APX decrease H$_2$O$_2$ level in cell and increase the stability of membranes. In both cultivars, CAT activity and salt stress effect increased in parallel. In the presence of high salinity level (200 mM NaCl), higher CAT activity was observed in the cv. Om Rabia3 resulting a better cope with reactive oxygen species. Similar to our results, higher CAT activity was found in many salt tolerant cultivars such as sesame, maize and wheat (Azevedo Neto et al., 2006; Koca et al., 2007).

It is worth to note that the enzyme POD increased more its activity in the tolerant cultivar in response to salt stress. Consequently, the low amount of H$_2$O$_2$ in the cv. Om Rabia3 under high salt stress condition could be attributed to an increased detoxification capacity mediated by the POD2 isoform activity (Fig. 7). Taken together, it seems that the salt tolerance phenotype of the cv. Om Rabia3 could be due to a low production of ROS or to its better ability to counteract ROS than the salt sensitive cultivar.

In this study, we show that the two Tunisian durum wheat cultivars respond differently to salt stress. Salt leads to oxidative stress and it is an abiotic elicitor of antioxidative defenses. The better development of the cv. Om Rabia3 plant results from the greater efficiency of the antioxidative response under NaCl stress conditions. In fact, in this cultivar the antioxidant enzyme system is enhanced more efficiently compared to the salt sensitive cultivar with higher capacity to accumulate ascorbate. Consequently, under saline conditions, the growth inhibition of this cultivar is retarded with low lipid damage. Thus, it is important to understand the genetics of detoxification of ROS in order to enhance crop salt tolerance.

References

Aebi H, 1984. Catalase in vitro. Method Enzymol 105: 121-126. https://doi.org/10.1016/S0076-6879(84)50516-3
Ahmad P, Sarwat M, Sharma S, 2008. Reactive oxygen species, antioxidants and signaling in plants. J Plant Biol 51: 167-173. https://doi.org/10.1007/BF03030694
Alscher RG, Erturk N, Heath LS, 2002. Role of superoxide dismutases in controlling oxidative stress in plants. J Exp Bot 53: 1331-1341. https://doi.org/10.1093/jexbot/53.372.1331
Ashraf M, 2009. Biotechnological approach of improving plant salt tolerance using antioxidants as markers. Biotechnol Adv 27: 84-93. https://doi.org/10.1016/j.biotechadv.2008.09.003
Athar HUR, Khan A, Ashraf M, 2008. Exogenously applied ascorbic acid alleviates salt-induced oxidative stress in wheat. Environ Exp Bot 63: 224-231. https://doi.org/10.1016/j.envexpbot.2007.10.018
Azevedo Neto AD, Prico JT, Eneas-Filho J, Braga de Abreu CE, Gomes-Filho E, 2006. Effect of salt stres on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. Environ Exp Bot 56: 235-241. https://doi.org/10.1016/j.envexpbot.2005.01.008
Ben Amor N, Ben Hamed K, Debez A, Grignon C, Abdelly C, 2005. Physiological and antioxidant responses of the perennial halophyte Crithium maritimum to salinity. Plant Sci 168: 889-899. https://doi.org/10.1016/j.plantsci.2004.11.002
Bowler C, Slooten L, Vandenbranden S, Rycke RD, Botterman J, Sybesma C, Montagu MV, Inze D, 1991. Manganese superoxide dismutase can reduce cellular damage mediated by oxygen radicals in transgenic plants. EMBO J 10: 1723-1732.
Bradford MM, 1976. A rapid and sensitive method for the quantification of microgram quantities of proteins utilizing the principle of protein-dye binding. Anal Biochem 72: 248-254. https://doi.org/10.1016/0003-2697(76)90527-3
Breusegem FV, Slooten L, Stassart JM, Moens T, Botterman J, Van Montagu M, Inze D, 1999. Overproduction of Arabidopsis thaliana FeSOD confers oxidative stress tolerance to transgenic maize. Plant Cell Physiol 40: 515-523. https://doi.org/10.1093/oxfordjournals.pcp.a029572
Brini F, Amara I, Feki K, Hanin M, Khouidi H, Masmoudi K, 2009. Physiological and molecular analyses of seedlings of two Tunisian durum wheat (Triticum turgidum L. subsp. Durum [Desf.]) varieties showing contrasting tolerance to salt stress. Acta Physiol Plant 31: 145-154. https://doi.org/10.1007/s11738-008-0215-x
Chaoui A, Mazhoudi S, Ghorbal MH, El Ferjani E, 1997. Cadmium and zinc induction of lipid peroxidation and effects on antioxidant enzyme activities in bean (Phaseolus vulgaris L.). Plant Sci 127: 139-147. https://doi.org/10.1016/S0168-9452(97)00115-5
Choudhury S, Panda P, Sahoo L, Panda SK, 2013. Reactive oxygen species signaling in plants under abiotic stress. Plant Signal Behav 8:23681 https://doi.org/10.4161/psb.23681
Desikan R, Mackerness S, Hancock JT, Neill SJ, 2001. Regulation of the Arabidopsis transcriptome by oxidative stress. Plant Physiol 127: 159-72.
Distelbarth H, Nägele T, Heyer AG, 2012. Responses of two naturally occurring sunflower mutants to high light. Plant Signal Behav 8:23681 https://doi.org/10.4161/psb.23681
ено"
Dvorák J, Noaman MM, Goyal S, Gorham J, 1994. Enhancement of the salt tolerance of Triticum turgidum L by the Kna1 locus transferred from Triticum aestivum L. chromosome 4D by homoelogous recombination. Theor Appl Genet 87: 872-877. https://doi.org/10.1007/BF00221141

Ellouzi H, Ben Hamed K, Cela J, Munné-Bosch S, Abdelly C, 2011. Early effects of salt stress on the physiological and oxidative status of Cakile maritima (halophyte) and Arabidopsis thaliana (glycophyte). Physiol Plant 142: 128-143. https://doi.org/10.1111/j.1399-3054.2011.01450.x

Epstein E, 1972. Mineral nutrition of plants: Principles and perspectives. John Wiley & Sons, NY.

Feki K, Kamoun Y, Mahmoud RB, Farhat-Khemakhem A, Gargouri A, Brini F, 2015. Multiple abiotic stress tolerance of the transformants yeast cells and the transgenic Arabidopsis plants expressing a novel durum wheat catalase. Plant Biochem Biochem 97: 420-431. https://doi.org/10.1016/j.plaphy.2015.10.034

Feki K, Farhat-Khemakhem A, Kamoun Y, Saibi W, Gargouri A, Brini F, 2016. Responses of transgenic Arabidopsis plants and recombinant yeast cells expressing a novel durum wheat manganese superoxide dismutase TdMnSOD to various abiotic stresses. J Plant Physiol 198: 56-68. https://doi.org/10.1016/j.jplph.2016.03.019

Foyer CH, Noctor G, 2011. Ascorbate and glutathione: the heart of the redox hub. Plant Physiol 155: 2-18. https://doi.org/10.1104/pp.110.167569

Foyer CH, Souriau N, Perret S, Lelandais M, Kunert KJ, Pruvost C, Jouanin L, 1995. Overexpression of glutathione reductase but not glutathione synthetase leads to increases in antioxidant capacity and resistance to photoinhibition in poplar trees. Plant Physiol 109: 1047-1057. https://doi.org/10.1104/pp.109.3.1047

Giannopolitis CN, Ries SK, 1977. Superoxide dismutases II: Purification and quantitative relationship with water soluble protein in seedlings. Plant Physiol 59: 315-318. https://doi.org/10.1104/pp.59.2.315

Gómez JM, Hernández JA, Jiménez A, del Rio LA, Sevilla F, 1999. Differential response of antioxidant enzymes of chloroplasts and mitochondria to long-term NaCl stress of pea plants. Free Radic Res 31: 11-18. https://doi.org/10.1080/10715769900301261

Gorham J, Wyn Jones RG, Bristol A, 1990. Partial characterization of the trait for enhanced K'-Na+ discrimination in the D genome of wheat. Planta 180: 590-597. https://doi.org/10.1007/BF02411458

Gossett DR, Millhillon EP, Lucas MC, Banks SW, Marney MM, 1994. The effects of NaCl on antioxidant enzyme activities in callus tissue of salt-tolerant and salt-sensitive cultivars of cotton. Plant Cell Rep 13: 498-503. https://doi.org/10.1007/BF00232944

Greenway H, Munns R, 1980. Mechanisms of salt tolerance in nonhalophytes. Annu Rev Plant Physiol 31: 149-190. https://doi.org/10.1146/annurev.pp.31.060180.001053

Hernández JA, Ferrer MA, Jiménez A, Barcelo AR, Sevilla F, 2001. Antioxidant systems and O2-/H2O2 production in the apoplast of pea leaves: its relation with salt-induced necrotic lesions in minor veins. Plant Physiol 127: 817-831. https://doi.org/10.1104/pp.001088

Hossain MA, Bhattacharjee S, Armin SM, Qian P, Xin W, Li HY, Burritt DJ, Fujita M, Tran LSP, 2015. Hydrogen peroxide priming modulates abiotic oxidative stress tolerance: insights from ROS detoxification and scavenging. Front Plant Sci 420: 16-6. https://doi.org/10.3389/fpls.2015.00420

Husain S, Munns R, Condon AG, 2003. Effect of sodium exclusion trait on chlorophyll retention and growth of durum wheat in saline soil. Aust J Agric Res 54: 589-597. https://doi.org/10.1071/AR03032

Jabs T, Dietrich RA, Dangl JL, 1996. Initiation of runaway cell death in an Arabidopsis mutant by extracellular superoxide. Sci 273: 1853-1856. https://doi.org/10.1126/science.273.5283.1853

Koca H, Bor M, Özdemir F, Türkan I, 2007. The effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. Environ Exp Bot 60: 344-351. https://doi.org/10.1016/j.envexpbot.2006.12.005

Lee DH, Kim YS, Lee CB, 2001. The inductive responses of the antioxidant enzymes by salt stress in the rice (Oryza sativa L.). J Plant Physiol 158: 737-745. https://doi.org/10.1016/S0176-6518(01)00086-0

Li Y, Chen Q, Liu Q, Zhang W, Ding R, 2003. Exogenous silicon (Si) increases antioxidant enzyme activity and reduces lipid peroxidation in roots of salt-stressed barley (Hordeum vulgare L.). J Plant Physiol 160: 1157-1164. https://doi.org/10.1016/j.jpلاs.2005.01.016

Maehly AC, Chance B, 1954. The assay of catalase and peroxidase. Meth Anal Biochem 1: 357-424.

Mandhania S, Madan S, Sawhney V, 2006. Antioxidant defense mechanism under salt stress in wheat seedlings. Biol Plant 227: 227-231. https://doi.org/10.1007/s10535-006-0011-7

Mhamdi A, Queval G, Chaouch S, Vanderauwera S, Van Breusegem F, Noctor G, 2010. Catalase function in plants: a focus on Arabidopsis mutants as stress-mimic models. J Exp Bot 61: 4197-4220. https://doi.org/10.1093/jxb/erq282

Mittler R, Zilinskas BA, 1992. Molecular cloning and characterization of a gene encoding pea cytosolic ascorbate peroxidase. J Biol Chem 267: 21802-21807.

Mittler R, Vanderauwera S, Gollery M, Van Breusegem F, 2004. Reactive oxygen gene network of plants. Trends Plant Sci 9: 1360-1385. https://doi.org/10.1016/j.tplants.2004.08.009

Mittova V, Tal M, Volokita M, Guy M, 2003. Up-regulation of the leaf mitochondrial and peroxisomal antioxidative systems in responses to salt-induced oxidative stress in the wild salt-tolerant tomato species Lycopersicon pennellii. Plant

Spanish Journal of Agricultural Research December 2017 • Volume 15 • Issue 4 • e0805
Comparison of an antioxidant system in tolerant and susceptible wheat seedlings

Cell Environ 26: 845-856. https://doi.org/10.1046/j.1365-3040.2003.01016.x

Mukherjee SP, Choudhuri MA, 1983. Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in Vigna seedlings. Physiol Plant 58: 166-170. https://doi.org/10.1111/j.1399-3054.1983.tb04162.x

Munné-Bosch S, 2005. The role of alpha-tocopherol in plant stress tolerance. J Plant Physiol 162: 743-748. https://doi.org/10.1016/j.jplph.2005.04.022

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

Noctor G, Foyer CH, 1998. Ascorbate and glutathione: keeping active oxygen under control. Annu Rev Plant Physiol Plant Mol Biol 49: 249-279. https://doi.org/10.1146/annurev.arplant.49.1.249

Pardo JM, 2010. Biotechnology of water and salinity stress tolerance. Curr Opin Biotechnol 21: 185-196. https://doi.org/10.1016/j.copbio.2010.02.005

Roxas VP, Smith JRK, Allen ER, Allen RD, 1997. Overexpression of glutathione S-transferase/glutathione peroxidase enhances the growth of transgenic tobacco seedlings during stress. Nat Biotechnol 15: 988-991. https://doi.org/10.1038/nbt1097-988

Sairam RK, Rao KV, Srivastava GC, 2002. Differential response of wheat genotypes to long-term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. Plant Sci 163: 59-66. https://doi.org/10.1016/S0168-9452(99)00197-1

Van Camp W, Capiau K, Van Montagu M, Inze D, Slooten L, 1996. Enhancement of oxidative stress tolerance in transgenic tobacco plants overproducing Fe-superoxide dismutase in chloroplasts. Plant Physiol 112: 1703-1714. https://doi.org/10.1104/pp.112.4.1703

Velikova V, Yordanov I, Edreva A, 2000. Oxidative stress and some antioxidant system in acid rain treated bean plants: Protective role of exogenous polyamines. Plant Sci 151: 59-66. https://doi.org/10.1016/S0168-9452(99)00197-1

Venkatesh J, Park SW, 2014. Role of L-ascorbate in alleviating abiotic stresses in crop plants. Bot Studies 55: 38. https://doi.org/10.1186/1999-3110-55-38

Wang J, Zhang H, Allen RD, 1999. Overexpression of an Arabidopsis peroxisomal ascorbate peroxidase gene in tobacco increases protection against oxidative stress. Plant Cell Physiol 40: 725-732. https://doi.org/10.1093/oxfordjournals.pcp.a029599

Willekens H, Inze D, Van Montagu M, Van Camp W, 1995. Catalase in plants. Mol Breed 1: 207-228. https://doi.org/10.1007/BF02277422

Wu G, Wei ZK, Shao HB, 2007. The mutual responses of higher plants to environment: physiological and microbiological aspects. Colloids Surf B Biointerfaces 59: 113-119. https://doi.org/10.1016/j.colsurfb.2006.09.004

Zhu D, Scandalios JG, 1994. Differential accumulation of manganese-superoxide dismutase transcripts in maize in response to abscisic acid and high osmoticum. Plant Physiol 106: 177-176. https://doi.org/10.1104/pp.106.1.173