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BIOCHEMICAL AND PHYSIOLOGICAL CHANGES IN RESPONSE TO SALINITY IN LEAVES AND ROOTS OF TWO DURUM WHEAT (*TRITICUM DURUM* DESF.) GENOTYPES

ABSTRACT

Salt stress is a major abiotic stress that limits agricultural productivity in many regions of the world. To understand the molecular basis of the salt stress response, two wheat (*Triticum durum* Desf.) cultivars, Karim and Azizi, which are of agronomic significance in Tunisia, were grown under non-saline and saline conditions (100 mM). Leaves and roots of control and salt-stressed plants were harvested after 11 days of salt treatment. Karim cultivar may behave as a typical Na^+ include, which compartmentalizes Na^+ within the leaf cell vacuoles where it could be used as an osmoticum to lower the osmotic potential necessary for the maintenance of the plant hydric status. While, accumulation of K^+ was greater in Karim cultivar compared to Azizi, in both organs, presenting an important manifestation of salinity tolerance. Significant changes in metabolism of antioxidative system were observed, with an increase in protein tyrosine nitration, which indicates that salinity stress induces a nitro-oxidative stress.

Keywords: durum wheat; oxidative stress; protein nitration; salinity stress.

Abbreviations:

- WC: water content
- CAT: Catalase
- SOD: Superoxide dismutase
- NOX: NADPH-oxidase
- GSH: Reduced glutathione
- GSNO: S-nitrosogluthatione

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- GSSG: Oxidized glutathione
- NO: nitric oxide
- NO_2: nitrogen dioxoide
- NO_2-Tyr: protein tyrosine nitration
- ONOO-: peroxynitrite
- ROS: reactive oxygen species
- RNS: reactive nitrogen species

INTRODUCTION

Salinity is a major abiotic stress adversely affects physiological and metabolic processes, leading to dimin-ished growth and yield (Azizpour *et al.*, 2010). Salt stress induces osmotic stress; the physiological drought, which typically reduces the growth (Munnes and Tester, 2008). Growth reduction due to sa-linity is also attributed to ion toxicity and nutrient imbal-ance, which causes not only high sodium (Na^+) and chlo-ride (Cl^-) accumulation in plants, but also antagonistically affects the uptake of essential nutrient elements as potassium (K^+), in competition with Na^+ (Sairam *et al.*, 2002; Zörb *et al.*, 2004).

Plant species adapt themselves to high salt concen-trations in the soils with the compartmentation of inor-ganic molecules in the vacuole (Chinnusamy *et al.*, 2005; Tejera *et al.*, 2006). Cations such as K^+ and Na^+ are known to be the major inorganic elements, which provide necessary osmotic potential for water uptake by plant cells (Tejera *et al.*, 2006). Regulation of K^+ uptake beside prevention of Na^+ entry and ef-flux of Na^+ from the cell, and furthermore, sequestration of Na^+ in vacuole for osmotic adjustment are the common strategies for maintenance of desirable K^+/Na^+ ratio in the cytosol (Chinnusamy *et al.*, 2005). Salt stress in addition to the known components of osmotic stress and ion toxicity, is also manifested as an oxidative stress (Esfandiari *et al.*, 2007).

One of the biochemical changes encountered in plants subjected to salinity is the production of reactive oxygen species (ROS) (Munns, 2002; Mittler, 2002; Gagneul *et al.*, 2007; Daneshmand *et al.*, 2010). Generation of these ROS causes significant damage to membranes and other essential macromolecules such as pigments, proteins, lipids and nucleic acids resulting in considerable cellular damage (Mittler, 2002; Sairam *et al.*, 2002; Ashraf, 2009). ROS are highly reactive and when the plant capacity for scavenging of those molecules is less than ROS production rate they can seriously disrupt normal metab-olism (Ashraf, 2009). However, plants possess defense mechanism in the form of antioxidants against the oxidative damage caused by ROS. This complex plant-antioxidant defenses system includes a number of enzymatic and non-enzymatic antioxidants to prevent or alleviate membrane damage caused by ROS. Catalase (CAT), superoxide dismutase (SOD) and NADPH-oxidase (NOX) are the major antioxidative enzymes that efficiently scavenge ROS and protect plant cells from these potential cytotoxic effects, with SOD probably being central in the defense

against toxic ROS (Marschener, 1995; Vranova *et al.*, 2002; Liang et al, 2003; Dalmia and Sawhney, 2004; Ashraf, 2009). Changes in activities of various antioxidant enzymes under salinity stress have been reported (Fahmy *et al.*, 1998, Hernandez *et al.*, 2000; Sairam *et al.*, 2002, 2005; Sairam and Srivastava, 2002).

Apart from these antioxidative enzymes, several small antioxidant molecules, such as glutathione and ascorbic acid can quench many kinds of ROS (Foyer *et al.*, 2004). Reduced glutathione (GSH) is one of the major, soluble, low molecular weight antioxidants, as well as, the major non-protein thiol in plant cells and plays a major role in the protection of cell and tissue structures from oxidative injury (Foyer and Noctor, 2011). Within cells, free glutathione is present mainly in its reduced form (GSH) which could be oxidized to glutathione disulfide (GSSG) in normal conditions but also under oxidative stress. GSH can react with nitric oxide (NO) to generate S-nitrosogluthatione (GSNO) which is considered to function as a mobile reservoir of NO bioactivity in plants (Durner and Klessig, 1999; Noble *et al.*, 1999). Ascorbic acid (vitamin C) is a water-soluble antioxidant molecule that acts as a primary substrate in the cycle pathway for enzymatic detoxification of hydrogen peroxide (Navarro *et al.*, 2006). In addition, Ascorbate acts directly to neutralize superoxide radicals, singlet oxygen or superoxide (Conklin *et al.*, 1997). Several studies indicate that the acquisition of salt resistance might also be a consequence of improving resistance to oxidative stress (Gueta-Dahan *et al.*, 1997). A correlation between antioxidant capacity and salinity tolerance has been reported in several plant species such as citrus (Gueta-Dahan *et al.*, 1997), rice (Demiral and Turkan, 2004, 2005) and wheat (Meneguzzo *et al.*, 1999).

More recently, the discovery that plant cells can generate the free radical nitric oxide (NO) has opened new ways of research. The involvement of nitric oxide (NO) and related molecules like nitrogen dioxoide (NO_2), *S*-nitrosoglutatione (GSNO) or peroxynitrite (ONOO-), designated as reactive nitrogen species (RNS), seems to be complementary in the mechanism of response of plants against environmental stress and accumulation of these species can result in nitrosative stress (Valderrama *et al.*, 2007; Corpas *et al.*, 2008, 2011,2013; Chaki *et al.*, 2009, 2011). Consequently, an environmental stress can cause an overproduction of NO and NO-related molecules and that can provoke toxic physiological consequences for plants. Thus, it is important to define a reliable marker of this type of stress (Corpas *et al.*, 2007). Nitration of protein tyrosines has been established as a potential biomarker for nitrosative stress in plants (Corpas *et al.*, 2007,2008; Valderrama *et al.*, 2007).

The objective of the present study was to elucidate the differential physiological and biochemical responses of two cultivars of durum wheat (Karim and Azizi) to salinity under hydroponic conditions. The results obtained will lead to a better understanding of these responses under salt conditions and can be of value in programs conducted to breed salt tolerant crop varieties and these attributes can also be introduced in species of interest through genetic engineering.

MATERIAL AND METHODS

Plant material and growth conditions

Seeds of two *durum* wheat (*Triticum durum* Desf.) cultivars, Karim and Azizi, were kindly provided by the "Institut National Agronomique de Tunis" (INAT) in Tunisia. The seeds were sterilized with a solution of sodium hypochlorite for 20 minutes, washed several times with distilled water and then germinated in Petri dishes on moist filter paper at 25°C in the dark for 3 days. Healthy seedlings were transferred for 3 days to continuously aerated nutrient solutions containing 1 mM KNO_3, 3.5 mM $Ca(NO_3)_2$, 1 mM KH_2PO_4, 1 mM $MgSO_4$, 0.5 mM K_2HPO_4, 32.9 µM Fe-K-EDTA, and micronutrients: 30 µM H_3BO_4, 5 µM $MnSO_4$, 1 µM $CuSO_4$, 1 µM $ZnSO_4$, 1 µM $(NH_4)_6Mo_7O_{24}$. The nutrient solution was renewed every 4 days and continuously aerated by electrical pumps. Plants were grown in a growth chamber: 26°C/40% relative humidity during the light period and 20°C/50% relative humidity during the dark period; photoperiod of 16 h daily with a light irradiance of 150 µmol.m^{-2}.s^{-1} at the plant canopy. Then, salt stress treatment was applied by adding 100 mM NaCl for 11 days. A nutrient solu-tion without the addition of NaCl served as the control.

Crude extracts of plant tissues: Wheat leaves (without stems) and roots were frozen in liquid N_2 and ground in a mortar with a pestle. The resulting coarse powder (1g) was transferred to 1/3 (w/v) extraction buffer containing 50 mM Tris-HCl, pH 7.8, 0.1 mM EDTA, 5 mm dithiothreitol (DTT), 0.1% (v/v) Triton X-100 and 10% (v/v) glycerol. Homogenates were centrifuged at 27,000 × *g* for 25 min at 4 °C, and the supernatants were used for the assays.

Water content : The tissue water content — *WC* (ml × g^{-1} DW) was determined according to the formula:

$$WC = \frac{FW - DW}{DW}$$

where;

DW — Dry weight, *FW* — Fresh weight

Ion analysis: Inorganic ions were extracted from dry matter with 0.5 N H_2SO_4 at room temperature for 48 h. K^+ and Na^+ were analyzed by flame emission using a spectrophotometer (Eppendorf, GmbH Hamburg, Germany).

Enzyme activity assay: Catalase activity (EC 1.11.1.6) was determined by measuring the disappearance of H_2O_2, as described by Aebi (1984).

SOD and NADPH oxidase isozymes: Native polyacrylamide gel electrophoresis was performed using acrylamide gels (10%) as described by Davis (1964).

- SOD (EC 1.15.1.1) isozymes were assayed in gels and visualized by a photochemical NBT (nitroblue tetrazolium) reduction method (Beauchamp and Fridovich, 1971). To identify the type of SOD isozymes, gels were incubated separately for 30-45 min in 50 mM K-phosphate pH 7.8, in the presence of NBT and then in the presence of riboflavine (Corpas *et al.*, 1998).

- NADPH oxidase (NOX; EC 1.6.3.1.1) isozymes activity were assayed in gels by the NBT reduction method of López-Huertas *et al.*, (1999), as modified by Sagi and Fluhr (2001).
- Gels were incubated in the dark for 20 min in a reaction mixture solution containing 50 mM Tris-HCl buffer pH 7.4, 0.2 mM NBT, 0.1 mM $MgCl_2$ and 1 mM $CaCl_2$. NADPH (0.2 mM) was added and the appearance of blue formazan bands was monitored. The reaction was stopped by immersion of the gels in distilled water.

Detection and quantification of GSH, GSSG, GSNO and ascorbate by liquid chromatography-electrospray/mass spectrometry (LC-ES/MS)

Wheat leaves and roots samples (400 mg) were ground along-side 1 ml of 0.1 M HCl using a mortar and pestle. Homogenates were centrifuged at 15,000 × *g* for 20 min at 4°C. The supernatants were collected, filtered through 0.22-µm polyvinylidene fluoride filters and immediately analyzed. All procedures were carried out at 4 °C and protected from light to avoid potential degradation of the analytes (GSH, GSSG, GSNO and ascorbate). The LC-ES/MS system consisted of a Waters Allience 2695 HPLC system connected to a Micromass Quattro Micro API triple quadrupole mass spectrometer, both of which were obtained from the Waters Corporation. HPLC was carried out using an Atlantis®T3 3µm 2.1mm x 100 mm column supplied by the same company. The Micromass Quattro Micro API mass spectrometer was used in positive electrospray ionization mode for simultaneous detection and quantification of GSH, GSSG, GSNO and ascorbate (Airaki *et al.*, 2011).

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and Western blotting

SDS–PAGE was carried out according to the method of Laemmli (1970) in 10% acrylamide slab gels. For Western blot analysis, proteins were transferred to PVDF membranes with a semi-dry Trans-Blot cell (Bio-Rad, Hercules, CA, USA). After transfer, membranes were used for cross-reactivity assays with a rabbit polyclonal antibody against 3-nitrotyrosine (NO_2-Tyr) (Corpas *et al.*, 2008) diluted 1:1000. For immuno-detection, an affinity-purified goat anti-(rabbit IgG)-horseradish peroxidase conjugate (Bio-Rad) and an enhanced chemiluminescence kit (ECLPLUS, Amersham, Piscataway, NJ, USA) were used.

Protein determination; Protein concentration was determined with the aid of the Bio-Rad Protein Assay (Hercules, CA, USA), using BSA as a standard.

Statistical analysis

All physiological and biochemical parameters were re-corded with five replications and means ± confidence limits at $P < 0.05$ level. The data are presented with standard error (SE) for at least five replicates (plants).

RESULTS

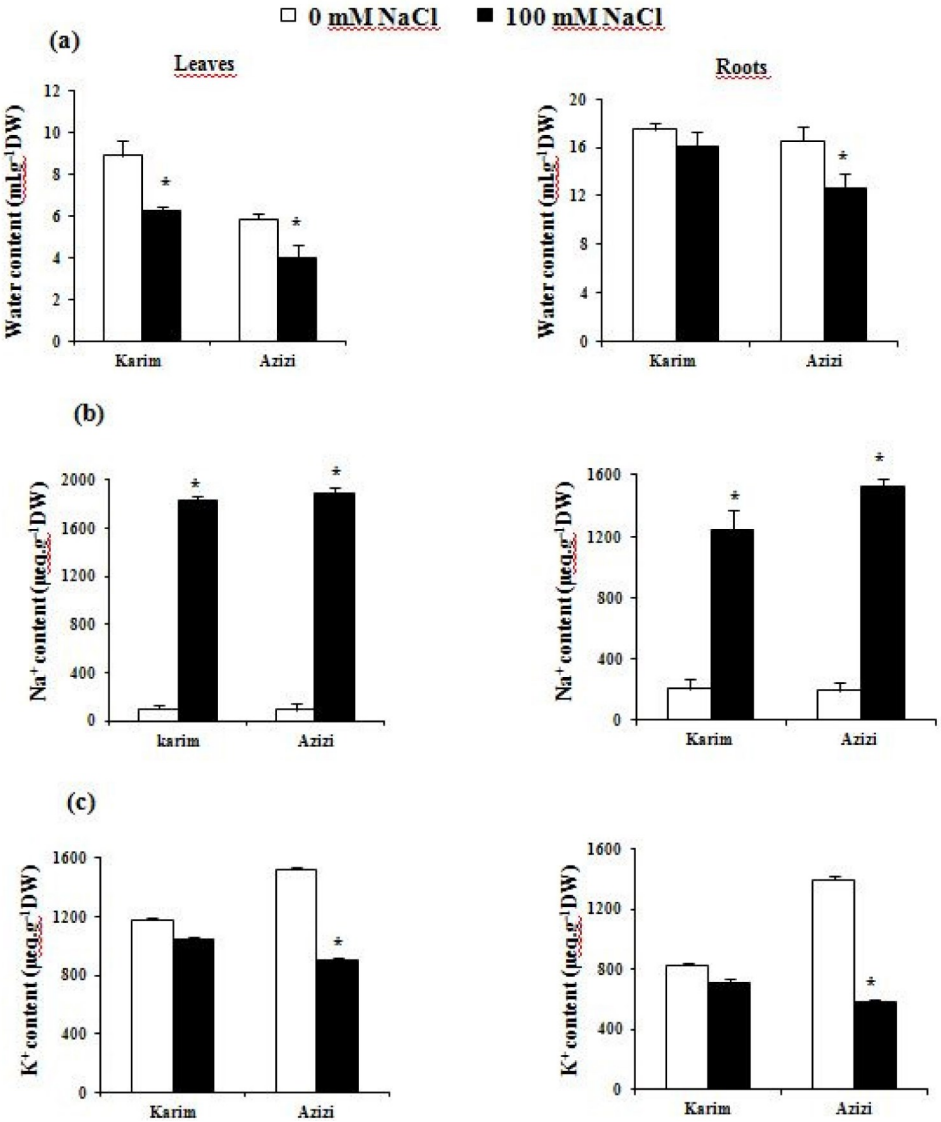

Fig. 1. Changes in (a) water content (ml×g^{-1} DW), (b) sodium and (c) potassium contents (µeq×g^{-1} DW) in leaves and roots of two wheat cultivars treated with 0 and 100 mM NaCl. Asterisks indicate that differences from control values were statistically significant at $P < 0.05$

Water content

Salt stress reduced leaf and root water contents in both wheat cultivars. In leaves, Karim cultivar maintained relatively higher WC under salt stress compared to Azizi (Fig. 1a). The roots water state was not affected by salinity for Karim.

Ion analysis

An increase in leaves and roots Na^+ concentration of both cultivars was observed under salt stress (Fig. 1b). The Na^+ concentration in leaves was greater than in roots for both cultivars. The two varieties present a higher transport of Na^+ towards the aerial parts.

K^+ concentration was markedly decreased in Azizi cultivar under salt stress by 40% and 58% in leaves and roots, respectively, compared with control plants (Fig. 1c). For Karim cultivar, the K^+ content proved to be insensitive under salt stress for both organs.

Catalase activity

The results showed that under salinity condition, there was a consistent increase in CAT activity in both cultivars. This increase reached 45% and 25% in leaves and roots, respectively, compared to the control, in Karim cultivar (Fig. 2). At the same time, this increase was more pronounced in leaves of Azizi cultivar and reached 86%, compared to the control. In contrast, no consistent pattern of increase or decrease in CAT activity was observed in roots of Azizi cultivar (Fig. 2).

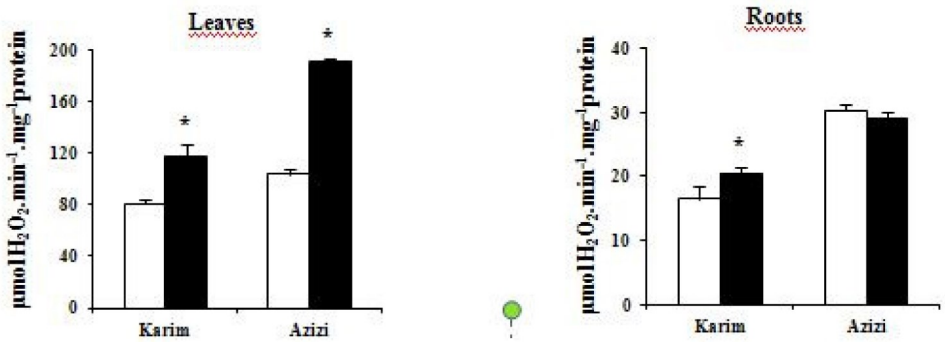

Fig. 2.Activity of catalase in leaves and roots of two wheat cultivars treated with 0 and 100 mM NaCl. Asterisks indicate that differences from control values were statistically significant at $P < 0.05$

Superoxide dismutase activity

Fig. 3a shows the analysis of superoxide dismutase (SOD) activity in native gel in leaves and roots of Karim and Azizi cultivars. One MnSOD, one Fe-SOD and two CuZn-SODs (I and II) were identified. In leaves, salt stress did not affect any of these SOD isoforms of Azizi cultivar. However, the bands from Ka-

rim NaCl-treated (K100) were strongly induced by salinity than that from the control plants. In roots, no significant changes were observed in the activity of three SOD isozymes of both cultivars under salinity.

NADPH-oxidase activity

NADPH oxidase (NOX) activity is considered to be an important source of superoxide radicals in plants. The detection of NOX activity in leaves and roots of two wheat cultivars using non-denaturing gels revealed the presence of three isozymes (I, II and III) in order to their increasing mobility in gels (Fig. 3b).

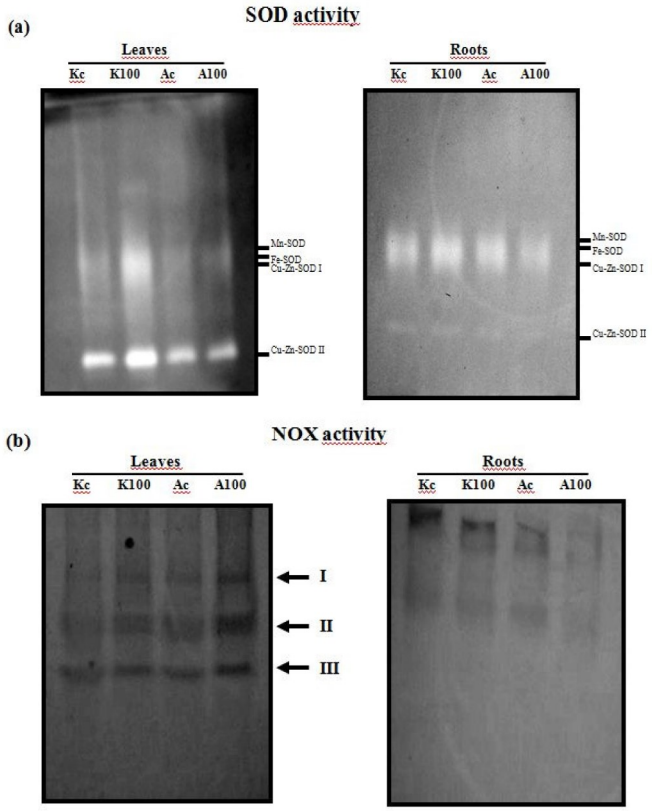

Fig. 3. (a) Superoxide dismutase (SOD) isoenzymes were separated by native-PAGE (10% acrylamide) and stained using a photochemical method (50 µg of protein per lane was loaded for leaves and 15 µg for roots) (b) Detection of NADPH oxidase (NOX) isoenzymes. Protein samples (50 µg protein per lane was loaded for leaves and 15 µg for roots) were separated by native-PAGE (10% acrylamide). The gels were then incubated with NBT and NADPH until the appearance of blue formazan bands was observed. (Kc: Karim control, K100: Karim treated with 100 mM NaCl, Ac: Azizi control, A100: Azizi treated with 100 mM NaCl)

As shown in Fig. 3b, three activity bands was detected in native gels in control and NaCl-treated wheat leaves for both cultivars, and the bands from Azizi NaCl-treated (A100) were obviously stronger than that from the control plants. However, there is no change observed in roots for both cultivars.

Content of glutathione (reduced, oxidized and S-nitrosylated) and ascorbate

Table 1 showed the status of non-enzymatic antioxidants, the content of ascorbate and glutathione (reduced, oxidized and S-nitrosylated) was studied using LC-ES/MS.

Table 1

Total GSH (reduced, oxidized, and S-nitrosylated) and ascorbate content in leaves and roots of two durum wheat cultivars treated with 0 and 100 mM NaCl. Asterisks indicate that differences from control values were statistically significant at P < 0.05

FW [Nmol×g^{-1}]	Durum wheat cultivars			
	Karim		Azizi	
	0 mM	100 mM	0 mM	100 mM
	Leaves			
GSH	206,47±1,2	36,59±1,5*	128±0,6	83,66±0,8*
GSSG	13,67±0,2	16,63±0,5*	13,56±0,3	11,62±0,1*
GSNO	8,77±0,1	6,47±0,06*	3,7±0,06	12,42±0,1*
Ascorbate	1779,47±4,2	13,26±0,5*	1702,75±7,5	1,54±0,01*
	Roots			
GSH	132,76±0,6	47,61±1,1*	134,88±1,1	70,9±0,5*
GSSG	13,51±0,1	11,33±0,1*	18,9±0,1	9,13±0,02*
GSNO	3,46±0,09	15,29±0,6*	2,67±0,05	16,05±0,2*
Ascorbate	1834,12±9,1	11,71±0,1*	919,51±7,5	4,74±0,06*

* – significant differences at P < 0.05

Under salinity stress conditions, ascorbate content decreased significantly, compared to the control, for both cultivars. However, GSH content decreased by 82% in leaves and 64% in roots for Karim cultivar. For Azizi cultivar, this decrease was less marked, reaching about 34% in leaves and 47% in roots, compared to the control. At the same time, GSSG content showed a significant reduction of 51%, only in roots of Azizi cultivar, in comparison with control. No significant changes were observed in GSSG content in Karim cultivar and leaves of Azizi cultivar, under salinity stress. S-nitroglutathione (GSNO) content showed an increase of 3.5-fold in leaves and 6-fold in roots of Azizi cultivar. While, for Karim cultivar, GSNO content decreased by 26% in leaves, in contrast, an increase of 4,5-fold was observed in roots, compared to the control.

Protein tyrosine nitration

Fig. 4 shows an immunoblot analysis of protein tyrosine nitration (NO_2-Tyr) in leaves and roots of two wheat cultivars exposed to 100 mM NaCl. A distinct protein nitration profile was found in leaves and roots. There was an increase in nitration of one immunoreactive bands of 45 kDa in leaves of both cultivars and this band was not detected in roots.

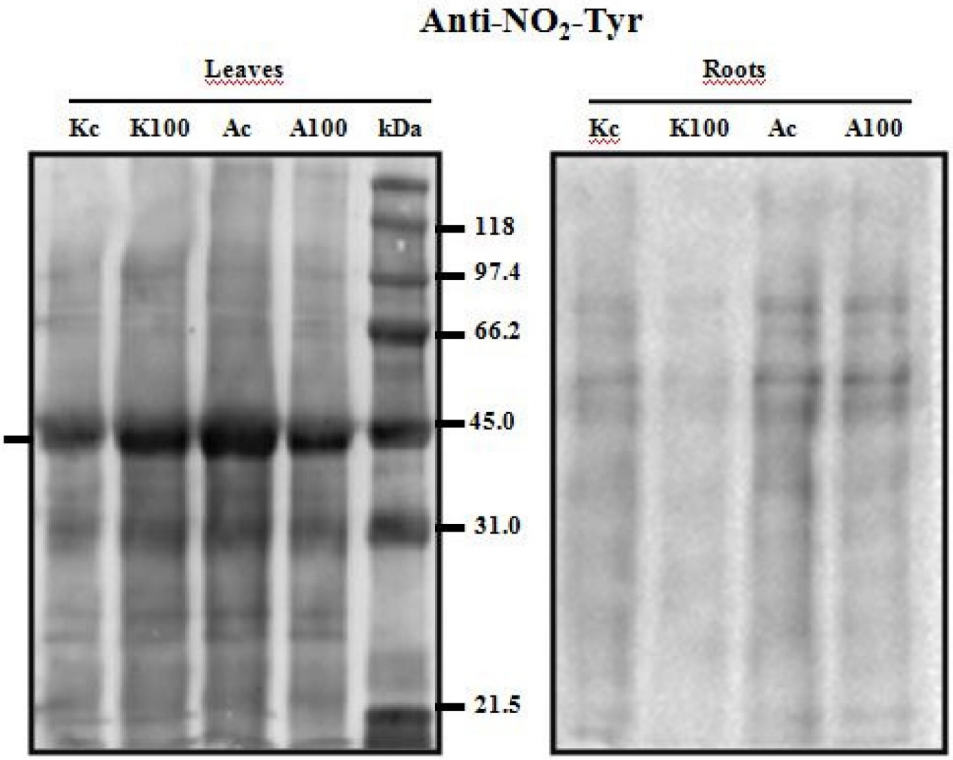

Fig. 4. Representative immunoblot showing protein tyrosine nitration (NO2-Tyr) in leaves and roots of two wheat cultivars treated with 0 and 100 mM NaCl. Leaf (50 µg protein per lane) and root (15 µg protein per lane) samples were subjected to SDS-PAGE, and western blotting analysis using an antibody against 3-nitrotyrosine (dilution 1:1000).The numbers on the right side of the immunoblot indicate the relative molecular masses of the protein markers. (Kc: Karim control, K100: Karim treated with 100 mM NaCl, Ac: Azizi control, A100: Azizi treated with 100 mM NaCl)

DISCUSSION

Salinity is a major abiotic stress reducing the yield of a wide variety of crops all over the world (Tester and Davenport, 2003). As a whole and taking into account the vast information provided by the literature, results presented in this paper confirm that the effects of salt stress are species-dependent. Salinity entails ionic stress, osmotic stress, and secondary stresses such as nutritional imbalances and oxidative stress for glycophytes (Zhu, 2002). Durum (pasta) wheat (*Triticum durum* Desf.) is more salt sensitive than bread wheat (*Triticum aestivum*), probably because of its poorer ability to exclude Na^+ from the leaf blade (Gorham et al, 1990). Even under irrigated conditions, durum wheat faces the problem of salt stress caused by

an excess of fertilization or by using salty water for irrigation (Ayers and Westcot, 1985; Katerji *et al.*, 2005).

The capacity of plants to tolerate high levels of salinity depends on the ability to exclude salt from the leaves, or to tolerate high concentrations of salt in the leaf (tissue tolerance). It is widely held that a major component of tissue tolerance is the capacity to compartmentalize salt into safe storage places such as vacuoles. This mechanism would avoid toxic effects of salt on photosynthesis and other key metabolic processes (James *et al.*, 2006).

In the present study, accumulation of Na^+ in the leaves and roots of both cultivars were significantly increased due to salt stress, this accumulation was more important in leaves than roots (Fig. 1b). Munns and James (2003) reported that several salt-tolerant tetraploid wheat genotypes accumulate very high leaf Na^+ levels. These genotypes may have a special ability to tolerate high internal levels of Na^+. The higher concen-tration of Na^+ may results from the greater capability for compartmentation of this ion in the vacuoles (Levigneron *et al.*, 1995). Karim cultivar may behave as a typical Na^+ include, which compartmentalizes Na^+ within the leaf cell vacuoles where it could be used as an osmoticum to lower the osmotic potential necessary for the maintenance of the plant hydric status (Fig 1a). In leaves, Karim cultivar maintained relatively higher WC under salt stress compared to Azizi (Fig. 1a) and the roots water state was not affected by salinity. While, accumulation of K^+ was greater in Karim cultivar compared to Azizi, in both organs (Fig. 1c), presenting an important manifestation of salinity tolerance. Azizi cultivar limits the potassium absorption, an essential ion for plant growth and development.

The decrease in the content and the activity of various antioxidants in response to salt stress has been reported in several species (Al Hakimi and Hammeda, 2001; Athar *et al.*, 2008; Fercha, 2011) and regarded as one of the mechanisms explaining, at least in part, the deleterious effects of salinity on crops. Plants use two systems to defend against and repair damage caused by oxidizing agents: The enzymatic antioxidant system, which is mainly represented by CAT, SOD and NOX. Then, the non enzymatic system, represented by fat-soluble low molecular weight compounds such as vitamin C and glutathione, etc. (Harinasut *et al.*, 2000).

In view of different researchers, salt tolerance is often correlated with a more efficient antioxidative system (Bor *et al.*, 2003; Ashraf and Harris, 2004; Demiral and Turkan, 2004; Sairam *et al.*, 2005). It is now generally known that salt stress causes the production of a variety of oxidants in plants. Most salt tolerant plants either produce lower amounts of ROS than those produced by salt sensitive plants, or they are more capable of counteracting ROS than salt sensitive plants (Mandhania *et al.*, 2006; Sairam *et al.*, 2005; Turkana and Demiral, 2009; Ashraf *et al.*, 2012) by a variety of enzymatic and non-enzymatic compounds (Apel and Hirt, 2004).

Fig. 2 showed that under salinity condition, there was a consistent increase in CAT activity in both cultivars. Similar behavior of increased activity by salinity stress in leaves and roots were found (Sairam *et al.*, 2005;

Manai *et al.*, 2014). However, higher activity of CAT and levels of induction were found in leaves compared with roots of salt-stressed plants.

In the present study, wheat leaves contain three different isoforms of SOD (Mn-, Fe-, Cu/Zn-SOD) (Fig. 3a). The remarkable induction of the SOD isoforms in leaves of salt-treated Karim cultivar enhances the relevance of this enzymatic system in the response of plants to salinity stress, as has been found earlier (Hernandez *et al.*, 1995; Valderrama et al,. 2006; Mittova *et al.*, 2002). However, in wheat leaves, Dalal and Chopra (2001) have identified four SOD isozymes in the crude extracts by native PAGE; three Cu/Zn SODs and one MnSOD.

As shown in Fig. 3b, three activity bands was detected in native gels of NADPH-oxidase in control and NaCl-treated wheat leaves for both cultivars, and the bands from Azizi NaCl-treated (A100) were obviously stronger than that from the control plants. This result further demonstrated that NOX is involved in the accumulation of reactive oxygen species (Hao et al, 2006).

In our study, using a new LC-ES/MS method (Airaki *et al.*, 2011), under salt stress, the content of both metabolites (GSH and ascorbate) was observed to have been significantly reduced (Table1), in both cultivars, suggesting that both are involved in the mechanism of response to salt stress. Miller *et al.* (2008) have reported that antioxidants such as ascorbate and glutathione play an important role in the regulation of the cellular ROS homeostasis. GSSG content was found to be not changed in both cultivars (Table 1). However, salt stress induced increase in GSNO content in both cultivars (Table 1). This raise was more important in Azizi cultivar, compared to the control. Altered activities of antioxidative enzymes and metabolites have commonly been reported and are used as indicators of oxidative stress in crops (Mittler, 2002).

Nitric oxide (NO), a free radical generated in plant cells, belongs to a family of related molecules designated as reactive nitrogen species (RNS). When an imbalance of RNS takes place for any adverse environmental stress, some of these molecules can cause direct or indirect damage at the cellular or molecular level, promoting a phenomenon of nitrosative stress (Corpas *et al.*, 2011). This was accompanied by a rise in protein tyrosine nitration, which is regarded as a marker of nitrosative stress (Corpas *et al.*, 2007), as has been described under salinity (Valderrama *et al.*, 2007). In this sense, the differences observed in tyrosine nitration between leaves and roots suggest that the mechanism of response against NaCl in both wheat cultivars must be different (Fig. 4). Protein tyrosine nitration was increased by salinity stress in both leaves and roots, but was more pronounced in leaves. Unlike roots, in leaves there was a strong induction in nitration of at one protein. This result possibly suggests that the leave proteins either become more susceptible to nitration or nitration of these proteins plays a role in protection from nitro-oxidative stress. Several reports suggest that different environmental stresses, which provoke oxidative stress, also induce a rise of protein tyrosine nitration. This mechanism occurs in olive and *Arabidopsis* under salt stress conditions (Valderrama *et*

al., 2006; Corpas *et al.*, 2009), in pea and sunflower seedlings by mechanical wounding (Corpas *et al.*, 2008; Chaki *et al.*, 2011), in tobacco BY-2 suspension cells in response to biotic stress (Saito *et al.*, 2006), and in sunflower seedlings after infection by mildew (Chaki *et al.*, 2009).

CONCLUSION

In conclusion, the results showed that Karim cultivar was able to escape the ionic toxicity under saline conditions via scavenging of ROS molecules and concomitantly controlled Na^+ absorption and transloca-tion. Those capabilities potentiate the plants survival and productivity under stressful conditions. Primary change of the total plant metabolism subjected to any stress is emergence of free radicals in cytoplasm and other cell components. Change in activities and numerous molecular forms of antioxidant enzymes are one of the parameters of plant biological tolerance against constantly changing environmental conditions. In present investigation, antioxidants status was found to be different in the two wheat cultivars under NaCl stress and normal physiological conditions. From the above results, it can be concluded that salt induced increase in antioxidant enzymes activities which are important for providing protection against ROS. In general, the obtained results can provide a guideline for the selection of salt-tolerant wheat cultivars at growth stages and may be useful to breeders and plant physiologists and could be reckoned as an efficient tool for screening new or existing cultivars for their salt tolerance.

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