Salt stress reveals differential physiological, biochemical and molecular responses in *T. monococcum* and *T. durum* wheat genotypes

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Abstract Salt stress responses implicate a complex mechanism and differ from plant species to another. In this study, we analyzed the physiological, biochemical and molecular responses to salt stress of the diploid wheat (*T. monococcum*) and compared to the tetraploid wheat (*T. durum*). Our results showed that the diploid wheat cultivar (cv. Turkey) is relatively tolerant to different salt stress conditions than the tetraploid wheat cultivar (cv. Om Rabia3). This tolerance was manifested by significant germination, plant growth and uptake of water generating cell turgor and development. Moreover, total chlorophyll content was higher in the diploid wheat than that in the tetraploid wheat. The Na⁺ content in leaf blade of the cv. Om Rabia3 was significantly higher than that of the cv. Turkey, suggesting that the diploid cultivar accumulates less toxic sodium in the photosynthetic tissues. This mechanism could be explained by the recirculation of the toxic ions Na⁺ into the xylem sap by SOS1 protein, which coordinates with HKT-like proteins to reduce the accumulation of Na⁺ ions in leaf blade. Interestingly, the expression of the three genes *SOS1*, *HKT* and *NHX* was

enhanced under salinity especially in leaf blade of the cv. Turkey. Moreover, this wheat cultivar induced the antioxidative enzymes CAT and SOD activity more efficiently than the other cultivar.

Keywords Diploid wheat · Tetraploid wheat · Salt stress · Antioxidant enzyme · Sodium transport

Introduction

Salinity is considered as the most severe environmental hazards for plant growth and productivity worldwide (Qadir et al. 2014). Wheat is a salt sensitive glycophyte plant and soil salinity affects dramatically the growth and grain yield (James et al. 2002; Munns et al. 2003; Brini et al. 2009). The capacity for Na⁺ elimination and the preservation of high ratio K⁺/Na⁺ in shoot are related by salt tolerance phenotype in wheat. Indeed, the hexaploid bread wheat (*Triticum aestivum* L. ssp. *aestivum*) show salt tolerance phenotype compared to the tetraploid durum wheat (*Triticum turgidum* L. ssp. *durum* (Desf.)), through the elimination of Na⁺ from the photosynthetic tissues. This trait is conferred by the *kna1* gene on chromosome 4DL of the bread wheat (Dubcovsky et al. 1996; Dvorak et al. 1994). The difference in sodium elimination can be found in various wheat genotypes, wheat progenitors and wild relatives plants (Colmer et al. 2006). Recently, it has been shown that the growth of the different organ is a better index of relative salt tolerance of different cultivars of the *T. aestivum* specie (Sharma 2015). The synthetic allo-hexaploid wheat tolerates significantly salt stress due to inheritance of favorable traits from the tetraploid (*T. turgidum*) and diploid (*Aegilops tauschii*) parents. Moreover, the expression of the *High-Affinity K⁺ Transporter 1;5*

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(*HKT1;5*) gene is induced under salt stress conditions in this synthetic wheat (Yang et al. 2014). In natural hexaploid wheat, *HKT1;5* gene plays a crucial role in salt tolerance through the sodium exclusion from the xylem vessels (James et al. 2006a). It was demonstrated that this gene corresponds to the *Kna1* QTL (Byrt et al. 2007, 2014). Sodium exclusion is controlled by another gene, *Nax1* (*HKT1;4*), found on the A genome of durum wheat (Lindsay et al. 2004). HKT proteins are active at the cell plasma membrane and are divided in two subfamilies. The members of the first one are specific to Na^+ transport, while the members of the second subfamily transport both Na^+ and K^+ (Almeida et al. 2013; Véry et al. 2014; Mansour 2014; Ben Amar et al. 2014; Byrt et al. 2014; Suzuki et al. 2016). In *Arabidopsis*, AtHKT1;1 controls the amount of Na^+ reaching the photosynthetic tissues, with the direct coordination with the plasma membrane Na^+/H^+ antiporter SOS1 (Salt Overly Sensitive 1) (Berthomieu et al. 2003; Sunarpi et al. 2005; Pardo 2010; Mansour 2014). It was reported that SOS1 regulates sodium transport from the root to the photosynthetic tissues via xylem (Shi et al. 2002; Olías et al. 2009). Thus, SOS1 plays a pivotal role in maintaining cellular ions homeostasis under salinity, through its regulation by two other components of SOS pathway, Ser/Thr kinase SOS2 and EF-hand SOS3 (Quintero et al. 2002; Shi et al. 2000; Atienza et al. 2007). Recently, it was demonstrated that the expression of the two candidate genes *SOS1* and *HKT1;1* is different in the halophyte *S. dolichostachya* under salinity. Indeed, the expression of *SOS1* enhances Na^+ loading in the xylem and consequently its accumulation in shoot (Katschnig et al. 2015). The expression of this plasma membrane protein confers ionic stress tolerance of the transgenic plants (Atienza et al. 2007; Yue et al. 2012; Feki et al. 2014).

In plant cells, oxidative damage is a secondary stress produced by the generation of the reactive oxygen species (ROS), including the superoxide radical (O_2^-), singlet oxygen ($^1\text{O}_2$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($\text{OH}^\cdot$). At high concentration, ROS are harmful and cause degradation of proteins and are implicated in various pathways of importance metabolic (Choudhury et al. 2013). Thus, plant cells activate the detoxification mechanisms including the enzymatic and non-enzymatic systems to modulate ROS concentrations. The first enzyme implicated in ROS scavenging system is the superoxide dismutase (SOD), which dismutates O_2^- to H_2O_2 . There are usually three SOD isoenzymes, Cu/Zn, Fe and Mn-SOD. The two first isoenzymes are localized in chloroplasts and cytoplasm, while Mn-SOD is localized in the mitochondria (Bowler et al. 1994). H_2O_2 is among the toxic ROS and has harmful effects in plant cells. It is catalyzed by catalase (CAT) and various classes of peroxidase (POD) to H_2O and O_2 . Recently, the two proteins Mn-SOD and CAT were

isolated from *Triticum durum*, and their expression in *Arabidopsis* plants enhances tolerance to multiple abiotic stresses (Feki et al. 2015, 2016). However, in others studies, they showed that the expression of one of the three SOD isoenzymes didn't achieve stress tolerance of the transgenic plants (Pitcher et al. 1991; Payon et al. 1997).

Other enzymes that are essential in the ROS scavenging system and are implicated in the ascorbate–glutathione cycle are glutathione reductase (GR), monodehydroascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR) (Foyer and Noctor 2011). In response to oxidative stress, some non-enzymatic components are produced such as ascorbate, tocopherols and the osmoprotectant proline (Suzuki et al. 2012; Rejeb et al. 2014). It was reported in various studies that the high accumulation of the non-enzymatic components and the activation of the antioxidant enzymes are consequences of salt stress tolerance (Koca et al. 2007a; Ellouzi et al. 2011). On the other hand, the effect of salinity on the antioxidant enzymes activities was analyzed in the same wheat genotype (Sairam et al. 2005; Esfandiari et al. 2007). In this study, this is the first physiological, biochemical and molecular analysis of two different wheat genotypes, the tetraploid (AABB) *Triticum durum* and the diploid (AA) *Triticum monococcum*.

Materials and methods

Plant material, growth conditions and germination assay

In this study, we used seeds of durum wheat [*T. turgidum* L. subsp. *durum* (Desf.)] cultivar Om Rabia3, supplied by INRAT (Laboratoire de Physiologie Végétale, Tunisia), and seeds of diploid wheat [*Triticum monococcum* L.] cultivar Turkey, supplied by the International Center for Agriculture Research in the Dry Areas (ICARDA-Syria).

Seeds were surface sterilized as described by Brini et al. (2009). Four-day-old seedlings were transferred to a hydroponics system in half-strength Hoagland's solution (Epstein 1972) and incubated at 25 °C in a growth chamber under a 16 h light/8 h dark photoperiod and 60 ± 10% relative humidity and the medium was changed every 6 days. Eighteen days after germination, salt stress treatments were applied. The NaCl concentration of the culture medium was augmented progressively to a final concentration of 50, 100 and 200 mM. For 100 and 200 mM treatments, 50 mM NaCl was added in the medium twice within 2 days and four times within 3 days, respectively.

Germination assay was performed using 30 seeds of each cultivar and at different salt concentrations (0, 50, 100 and 200 mM NaCl).

Leaf surface determination

UTHSCSA image tool is a free image processing and analysis program. It can acquire, display, edit and analyze images (<http://ddsdx.uthscsa.edu/dig/itdesc.html>). Total leaf area of wheat seedlings in hydroponics system was calculated in centimetres square using image tool program. Photograph of plants were taken every 2 days.

Relative water contents

To measure the relative water content (RWC), leaves were excised and their fresh weight taken immediately. After floating on deionised water at 4 °C overnight, their rehydrated weight was determined. Finally, they were dried in an oven at 70 °C overnight and weighed. The RWC was calculated as (fresh weight–dry weight)/(rehydrated weight–dry weight).

Total chlorophyll content determination

To determine the chlorophyll amount, fresh leaves (0.5 g) of leaves plants were crushed in 80 % acetone. Grinding was repeated several times to remove chlorophylls efficiently. The extract was centrifuged at 2500 g for 5 min. The absorbance of the obtained supernatants was measured at 646 and 663 nm. The total chlorophyll content in each sample, expressed in mg g^{-1} (FM), was calculated using the following formula: $[(7.15 \times \text{DO663}) + (18.71 \times \text{DO646})]V/M$, where V corresponds to the volume of the total extract per liter and M to the mass of the fresh material (FM).

Preparation of enzymatic extract and antioxidant enzyme activities

Aliquots of frozen fresh shoot material (0.5 g) were ground to a fine powder with liquid nitrogen and homogenized in a cold solution containing 100 mM Tris–HCl buffer (pH 8.0), 10 mM EDTA (Ethylenediaminetetraacetic acid), 50 mM KCl, 20 mM MgCl_2 , 0.5 mM PMSF (Phenyl methyl sulfonyl fluoride), and 2 % (w/v) PVP (Polyvinylpyrrolidone). The homogenate was centrifuged at $14,000 \times g$ for 30 min at 4 °C, and the supernatant was used for determination of the antioxidative enzyme activities.

Total catalase (CAT) activity was measured according to the method of Aebi (1984), by monitoring the decline in absorbance at 240 nm as H_2O_2 was consumed. An aliquot of crude enzyme extract was added to the reaction mixtures containing 50 mM phosphate buffer (pH 7), 30 mM H_2O_2 . One unit of CAT was defined as $1 \mu\text{mol ml}^{-1} \text{H}_2\text{O}_2$ decomposed per minute. Total superoxide dismutase (SOD) activity was determined by measuring the percentage of inhibition of the pyrogallol autoxidation (Marklund

and Marklund 1974). An aliquot of crude enzyme extract was added to 10 mM pyrogallol in Tris-cacodylic acid-diethylene triamine penta acetic acid buffer (pH 7.4–8). The rate of autoxidation was taken from the increase in OD of the reaction solution per min. One unit of SOD was defined as the enzyme quantity required causing 50 % inhibition of the rate of the pyrogallol autoxidation in comparison with tubes lacking the plant extract. The activities of SOD and CAT were expressed as units mg^{-1} protein.

Quantitative H_2O_2 determination

The concentration of H_2O_2 was measured following the method reported by Velikova et al. (2000). The fresh samples were homogenized in 0.1 % (w/v) trichloroacetic acid (TCA), and then centrifuged at $12,000 \times g$ for 15 min. An aliquot of the supernatant was added to 10 mM phosphate buffer (pH 7.0) and 1 M potassium iodide (KI). The mixture was then vortexed and its absorbance was read at 390 nm.

RNA extraction and Q-PCR assay

Total RNA from roots, leaf sheath and leaf blade of 2 week-old plants treated with 50 mM NaCl for 4 and 48 h was extracted using the RNeasy total RNA isolation kit (Qiagen). To remove contaminating DNA, RNAs ($10 \mu\text{g}$) were treated with RNase-free DNase (Promega). DNase-treated RNA samples ($0.5 \mu\text{g}$) were reverse-transcribed using M-MLV reverse transcriptase (Invitrogen). Real-time PCR was performed in 96-well plates with the CFX 96 Touch™ Real-Time PCR System (Biorad) using SYBR @ Select Master Mix for CFX (Applied Biosystems). PCR reactions were performed in a $10 \mu\text{l}$ final volumes containing 3 μl DNA (40 ng of cDNA), 0.5 μl of each primer (10 μM), 5 μl 2 $\times$ SYBR @ Select Master Mix and 1 μl of RNase-free water (Sigma). The reaction consisted of an initial denaturation at 94 °C for 10 min followed by 45 cycles at 94 °C for 10 s, 60 °C for 10 s and 72 °C for 15 s, then a melting curve (5 s at 95 °C, 1 min at 65 °C and 5 min with temperature increasing from 65 to 97 °C). Absence of primer dimer and specificity of amplification was confirmed by analysis of water controls and by examination of melting curves. The primers used for real-time qPCR were presented in Table 1. The relative expression was quantified by using the comparative CT method with the *Actin* gene as an internal expression standard (Livak and Schmittgen 2001). The relative expression level was calculated as follows: $2^{-\Delta\Delta C_T}$, where $\Delta\Delta C_T = (C_{T, \text{Target gene}} - C_{T, \text{Actin}})_{\text{stressed}} - (C_{T, \text{Target gene}} - C_{T, \text{Actin}})_{\text{control}}$. Three biological repetitions were performed to calculate the expression level.

Table 1 Sequences of nucleotides primers used in real time expression analysis

Primers	Nucleotide sequences (5'–3')	Accession no.
<i>Wheat actin</i>		
Act F	TGCATAGAGGGAAAGCACG	AB181991.1
Act R	AACCCAAAAGCCAACAGAGA	
<i>SOS1 gene</i>		
SOS F	AGAGTTTCCGGAGATCCAGC	EU552490.1
SOS R	TGCTGCCATACATGCTGACT	
<i>NHX gene</i>		
NHX F	GCAGCTGATATCTCAAGCCA	AY296910.1
NHX R	ACTGAAGGTTTGTGTTGGGGG	
<i>TmHKT1;4-A2 gene</i>		
TmHKT1;4-A2 F	GGAGACACAGGGTATCAGGC	EF062819.1
TmHKT1;4-A2 R	AGCTTCTCCCTCTCGGTGAC	
<i>TdHKT1;4-2 gene</i>		
TdHKT1;4-2 F	GATGCTAGCGGCAACGGT	KF443079.1
TdHKT1;4-2 R	CGCGTTTCGACACCTTCTC	
<i>CAT1 gene</i>		
CAT F	CGAGAAGATGGTGATCGAGAA	KP696753.1
CAT R	TGTTGATGAATCGCTCTTGC	
<i>MnSOD gene</i>		
SOD F	GCCATTGATGAGGATTTTGG	KP696754.1
SOD R	CAAAGCTAGCCACACCCATC	

Measurement of ions Na⁺ and K⁺ contents

Na⁺ and K⁺ ions were extracted by solubilizing the dried tissues in 0.1 N hydrochloric acid for 24 h. Na⁺ and K⁺ contents from plant tissues were determined by flame spectrophotometry (SpectrAA 220 FS, Varian).

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 salt stress on seeds germination, plants growth, water content and chlorophyll content

Seeds of diploid wheat *Triticum monococcum* L. (cv. Turkey) and tetraploid wheat *Triticum durum* Desf. (cv. Om Rabia3) genotypes were germinated in the presence of different salt concentration. In the absence of salt in the medium, the germination rate of the seeds of these two cultivars was similar. However, the presence of increasing concentrations of NaCl decreased the germination percentage of both wheat cultivars. This decrease was pronounced in the cv. Om Rabia3 which was about 80%,

Fig. 1 Seeds germination percentage of the diploid wheat *T. monococcum* (cv. Turkey) and the tetraploid wheat *T. durum* (cv. Om Rabia3) under different salt concentrations (0, 50, 100 and 200 mM NaCl). Values represent mean $\pm$ SE of four replicates. Asterisks indicate a statistically significant difference ($P < 0.05$)

whereas it was only 50% in the cv. Turkey in the presence of high salt concentration (200 mM NaCl) (Fig. 1). On the other hand, the presence of different salt concentrations in the growth medium affected dramatically the growth of the cv. Om Rabia3 compared to the other cultivar (Fig. 2a). These observations were confirmed by measuring the dry weight (DW), total leaf area (TLA) and roots length of

Fig. 2 a Effect of salt stress treatment on plant growth of two different wheat genotypes, the cv. Turkey and the cv. Om Rabia3. Determination of the dry weight (DW) of the different organ of these two wheat cultivars, **b** total leaf area (TLA), **c** and root length,

d under low (50 mM), medium (100 mM) and high (200 mM) salt stress treatment during 3 days. Values represent mean $\pm$ SE of six replicates. Asterisks indicate a statistically significant difference ($P < 0.05$)

these two cultivars exposed or not to these salt stress conditions. The maximal growth potential of the cv. Turkey was observed at moderate salinity (50 mM NaCl) (Fig. 2b). As shown in Fig. 2c, the same TLA values were obtained in the absence of salt stress. Nevertheless, TLA was reduced about 30 and 80% in the cv. Om Rabia3 treated with 100 and 200 mM NaCl, respectively. However, the reduction was about 15 and 50% in the other cultivar exposed to medium and high salt concentrations, respectively (Fig. 2c). It is worth to note that the difference between the roots length was observed when plants are exposed to 50 or 100 mM NaCl in the medium. Indeed, roots growth of the cv. Om Rabia3 was spectacularly reduced compared to the cv. Turkey (Fig. 2d). All these observations suggested that the two wheat cultivars respond differently to salt stress under germination and growth states.

As shown in Fig. 3a and b, RWC values of leaves of the unstressed seedlings were similar. The exposition to increased salt concentration reduced RWC values of the two wheat seedlings cultivars. However, RWC of the cv. Om Rabia3 decreased significantly compared to the other cultivar when plants are exposed either to medium salt concentration (100 mM NaCl) during 8 days or to high salt stress (200 mM NaCl) for 3 days. Moreover, root and leaf sheath water content decreased significantly with the increase of salt concentration in the two wheat genotypes. However, the difference between these two cultivars was observed when plants are exposed to medium salt stress for 8 days. On the other hand, leaf blade water content of the cv. Turkey was higher than that the other cultivar, when plants are treated with 200 mM NaCl for 3 days or with medium salt concentration for 8 days (Fig. 3c, d).

Total chlorophyll content was also affected by the exposition of these two wheat cultivars to the different salt concentration in the medium. In fact, at lowest salt concentration (50 mM NaCl), the chlorophyll content was different in these two cultivars and the cv. Turkey retained more chlorophyll at all salt levels (Fig. 4).

Na⁺ and K⁺ accumulation in the different organ of the two different wheat genotypes under salt stress treatment

The cv. Om Rabia3 accumulated significantly the sodium in roots under increasing salinity compared to the cv. Turkey. In this organ, Na⁺ concentrations ranged from 800 to 1300 $\mu\text{mol g}^{-1}$ DW at 50 and 200 mM NaCl, respectively. However, Na⁺ content was similar in leaf sheath of the two wheat cultivars exposed to low salt concentration (50 mM NaCl). It is worth to note that in this organ, Na⁺ accumulation reached to the maximum in the cv. Turkey,

and it was about 1.5-fold relative to the cv. Om Rabia3. However, at 200 mM NaCl, Na⁺ content decreased notably in the cv. Turkey and continued to increase in the other cultivar. In leaf blade, at low and medium salt concentrations, there are any differences in the sodium content between the two wheat genotypes. Nevertheless, high salt stress (200 mM NaCl) generated a significant fourfold increase in Na⁺ accumulation in the cv. Om Rabia3 relative to the other cultivar (Fig. 5a).

To investigate furthermore the storage of Na⁺ in the different organ of these wheat cultivars, we measured Na⁺ contents at different times of exposition to 100 mM NaCl. As shown in Fig. 5b, at different time of exposition to salt stress, the cv. Om Rabia3 significantly sequestered sodium in root, leaf sheath and leaf blade. Moreover, at a long-term of salt stress application (8 days), Na⁺ was accumulated significantly in leaf sheath compared to roots and leaf blade of the cv. Om Rabia3, and reached 3000 $\mu\text{mol g}^{-1}$ DW in leaf sheath and only 1500 $\mu\text{mol g}^{-1}$ DW in roots and leaf blade (Fig. 5b). Thus, these measurements indicated that these two wheat genotypes seem to have the divergent capacity to accumulate Na⁺ in the different organs.

On the other hand, K⁺ ions were considerably accumulated in roots and leaf sheath of the cv. Turkey treated with low and medium salt concentrations, as compared to the cv. Om Rabia3 (Fig. 5c). During the exposition of 100 mM NaCl, K⁺ content decreased and was similar in the different organ of the two cultivars (Fig. 5d).

Using Real-time PCR analysis, the expression of three candidate genes *SOS1*, *NHX* and *HKT* coding for ionic transporter was checked in roots, leaf sheath and leaf blades of the two cultivars Turkey and Om Rabia3 exposed to 50 mM NaCl. Compared to the control conditions, the expression of these three genes increased notably in roots and leaf sheath of the cv. Om Rabia3 after 48 h of stress application. Indeed, in roots, this increase was about 2fold for *SOS1* and *NHX* genes, and about 1.5-fold for *HKT* gene, relative to the other cultivar. In leaf sheath, it was about 3fold for *SOS1* and *NHX* genes and 5fold for *HKT* gene. However, the transcript levels of these genes were higher in the cv. Turkey, compared to the cv. Om Rabia3. In fact, *SOS1* gene expression reached the maximum during 4 h of stress treatment. Compared to the cv. Om Rabia3, the expression level of this gene in leaf blade was about 6fold and 4fold during 4 and 48 h of stress application, respectively. Concerning *NHX* gene, the expression was similar during the two times of stress application, and it was about 3fold relative to the basal expression. On the other hand, the maximum mRNA *HKT* gene expression was observed during 48 h of stress treatment, and it was 4fold relative the basal expression (Fig. 6).

Fig. 3 **a** Relative water content (RWC) of leaf blade of the two wheat cultivars (cv. Turkey and the cv. Om Rabia3) under different salt concentrations (0, 50, 100 and 200 mM NaCl) during 3 days, and under exposition with 100 mM NaCl during 2, 4 and 8 days, **b** determination of the water content of the different organ of these

wheat cultivars under salt stress treatment, **c** and under exposition to medium salt stress (100 mM) during 2, 4 and 8 days, **d** values represent mean $\pm$ SE of four replicates. Asterisks indicate a statistically significant difference ($P < 0.05$)

Fig. 4 Determination of the total chlorophyll content of the two wheat cultivars (cv. Turkey and the cv. Om Rabia3) under different salt concentrations (0, 50, 100 and 200 mM NaCl) and during 3 days. Values represent mean $\pm$ SE of three replicates. Asterisks indicate a statistically significant difference ($P < 0.05$)

Oxidative stress evaluation and analysis of the two antioxidant enzymes CAT and SOD in the leaf blade

The oxidative damage was evaluated by measuring the accumulation of H_2O_2 in the different organs of the stressed wheat cultivars. As shown in Fig. 7a, the application of salt stress for short-term induced significant accumulation of H_2O_2 content only in leaf blade of the cv. Om Rabia3. Nevertheless, H_2O_2 contents were stable during the salt stress treatment in leaf sheath of these two cultivars, and negligible in roots (Fig. 7a).

The activities of two essential antioxidant enzymes SOD and CAT were analyzed in leaf blade of these two wheat cultivars exposed 50 mM NaCl for 4 and 48 h as compared to control. Contrary to SOD activity, CAT activity increased significantly in the cv. Turkey, and it researched the maximum during 48 h of stress treatment. This increase was about 1.8-fold relative to the non-treated plants. However, CAT activity decreased notably in the cv. Om Rabia3 in this time of stress, and became lower than that in the other cultivar. On the other hand, SOD activity was reduced in the cv. Om Rabia3 by 20 and 30% during 4 and 48 h of stress, respectively, whereas, it was stable in the cv. Turkey (Fig. 7b, c). It is worth to note that the expression of *CAT* and *Mn-SOD* genes increased notably in the cv. Turkey with stress treatment, compared to the other cultivar (Fig. 8).

Discussion

Salt stress is among the abiotic stress issues worldwide that changes plant cultivation and productivity, especially in regions where saline water is used for irrigation. The

important mechanism in salt stress tolerance is the ability to remove the excess of Na^+ ion in leaves that produces a premature senescence of leaves (Pardo 2010). Contrary to durum wheat cultivars, this trait is present in bread wheat

Fig. 5 Na^+ and K^+ content of the different organs of two wheat cultivars treated with different salt concentration (0, 50, 100 and 200 mM NaCl) during 3 days, and exposed to medium salt stress (100 mM) during 2, 4 and 8 days. Na^+ (a, b) and K^+ (c, d) contents calculated as mg of ions per g of dry weight (DW) of the tissue. Sodium and potassium contents were determined in the roots, the leaf sheath and the leaf blade. Values represent mean $\pm$ SE of three replicates. Asterisks indicate a statistically significant difference ($P < 0.05$)

cultivars (Dubcovsky et al. 1996). Na^+ ion elimination is controlled by two key genes, *Nax1* and *Nax2*, mapped to the genome A (Munns et al. 2003). *Nax1* reduces the passage of Na^+ ion to the leaf blade through the enhancement of sodium retention in leaf sheath, whereas *Nax2* is involved in the reduction of Na^+ translocation from root to shoot (James et al. 2006a). The genome A is provided by the diploid species, *T. urartu* (AA), which shows a great Na^+ exclusion and K^+/Na^+ discrimination than durum wheat (Colmer et al. 2006). Recently, TmHKT1.4-A1 and TmHKT1.4-A2 from *T. monococcum*, were functionally characterized and the conductance of TmHKT1.4-A2 was higher than HKT1.4-type transporters from durum wheat (Tounsi et al. 2016). In this investigation, we found that the closely related A-genome specie *T. monococcum* ssp. *monococcum* (cv. Turkey) shows a relative salt stress tolerance phenotype compared to the tetraploid wheat *T. Turgidum* ssp. *durum*. This tolerance was evaluated using a cluster of physiological parameters. At the germination stage, the cv. Om Rabia3 is more sensitive to salt stress. Moreover, plant growth is less affected in the cv. Turkey than the other cultivar under different salt concentrations. At these conditions, the difference of RWC in these two wheat genotypes suggests that the cv. Turkey is able to maintain high water in leaves, and consequently maintenance of leaf growth and development. In parallel, it was demonstrated in many studies that growth reduction caused by salt stress could be associated with the osmotic stress, which influence cell development (Thiel et al. 1988; Ben Amor et al. 2005; Ellouzi et al. 2011). In addition, increased turgor can allow for a greater stomatal aperture, and consequently favor faster photosynthesis. It has been observed in previous study that chlorophyll degradation rate is high in salt sensitive wheat cultivars compared to the salt tolerant cultivars (Sairam et al. 2005), which is clearly in line with our findings.

Salt stress causes ionic stress by high accumulation of the toxic ion Na^+ in cytosol. The two different wheat genotypes used in this study showed different accumulation of Na^+ ion in their different organs. The higher accumulation was observed in a real part of the cv. Om Rabia3, which could explain his sensitivity to salinity compared to the cv. Turkey. It was shown previously that the cv. Om Rabia3 limits loading Na^+ to the leaf blade by its sheath

Fig. 6 Real-time RT-PCR analysis of *SOS*, *a* *NHX*, *b* and *HKT1;4*, *C* expression in different tissues (roots, leaf sheath and leaf blade) of the two wheat cultivars (cv. Turkey and the cv. Om Rabia3). Relative expression level was measured under normal condition or under 50 mM NaCl treatment for 4 and 48 h. Data in (a–c) are mean $\pm$ SE of three biological replicates. Asterisks indicate a statistically significant difference ($P < 0.05$)

storage capacity compared to other wheat cultivar that has the same genotype (Brini et al. 2009).

The major mechanism involved in salt stress tolerance is the transport of ions through plasma membrane (Volkov 2015). The two proteins HKT and SOS1 cooperate to remove the toxic sodium in the cytosol under salinity, and the NHX exchangers storage sodium in vacuole (Pardo

2010). These three proteins were identified in *T. durum* and it was shown that they are involved in salt stress response (Brini et al. 2009; Feki et al. 2011; Ben Amar et al. 2014). Recently, two HKT1;4 transporters from *T. monococcum* were characterized (Tounsi et al. Unpublished data). Interestingly, we demonstrated in this work that the expression of the genes coding for these proteins was enhanced preferentially in leaf blade of the salt tolerant *T. monococcum* wheat (cv. Turkey) under salinity, compared

to the salt sensitive *T. durum* wheat (cv. Om Rabia3). It is worth to note that *SOS1* transcript accumulation was higher in this organ under these stress conditions than *NHX* and *HKT* genes expression. Thus, the salt tolerance phenotype observed in *T. monococcum* is probably due to the reduction uptake of the toxic ion Na^+ and its loading into the xylem sap.

Under salt stress, ROS are produced at high levels and to equilibrate ROS amount, plant cells enhance the enzymatic antioxidant defense systems which include the three enzymes SOD, CAT and POD (Choudhury et al. 2013). Our results showed that H_2O_2 is produced dominantly in leaf blade of the cv. Om Rabia3 exposed to salt stress, whereas H_2O_2 level was almost unchanged in leaf blade of the cv. Turkey. In parallel, it was report in various studies that salt stress produced a significant accumulation of H_2O_2 in salt sensitive plants as compared to the salt tolerant plants (Ellouzi et al. 2011). The high level of H_2O_2 inhibits the Calvin cycle and consequently reduces CO_2 fixation (Yamazaki et al., 2003). Thus, plant cells reduce H_2O_2 levels through the implication of CAT and POD. The decrease in the content of H_2O_2 in cv. Turkey is the result of SOD reaction which is accompanied by an increased enzymatic capacity to decompose it by CAT. Therefore, SOD and CAT activities increased greatly and in parallel with salt stress. The correlation between salinity tolerance

Fig. 7 Effect of salt stress treatment (50 mM NaCl) on H_2O_2 content in roots, leaf sheath and leaf blade of the two wheat cultivars (cv. Turkey and cv. Om Rabia3) during 4 and 48 h. Values represent mean $\pm$ SE of four replicates. Asterisks indicate a statistically significant difference ($P < 0.05$)

Fig. 8 CAT and SOD activities, **a** and quantitative expression, **b** analysis in leaf blade of the two different wheat genotypes (cv. Turkey and the cv. Om Rabia3) exposed or low salt stress treatment

(50 mM NaCl) and control during 4 and 48 h. Values represent mean $\pm$ SE of three replicates. Asterisks indicate a statistically significant difference ($P < 0.05$)

and the increase of SOD and CAT activities has been demonstrated in many works (Ben Amor et al. 2005; Mandhania et al. 2006; Koca et al. 2007b; Ellouzi et al. 2011).

In *Arabidopsis* cells exposed to oxidative stress, some genes showed changes in expression levels. It was reported recently that the expression of *TdCAT1* and *Mn-SOD* genes from *T. durum* was up-regulated by various abiotic stress (Feki et al. 2015). Moreover, in *Arabidopsis*, the expression of *CAT1* gene is enhanced by different abiotic stress (Du et al. 2008). Our results showed that medium salt concentration produces an elevate expression of *CAT* and *MnSOD* genes in leaf blades of the cv. Turkey, compared to the cv. Om Rabia3. Taken together, it seems that the salt tolerance phenotype of the *T. monococcum* could be due to a low production of ROS or to its better ability to counteracting ROS than *T. durum*.

In conclusion, the results have shown a varied response in two different wheat genotypes, *T. durum* and *T. monococcum*, to salt stress. The salt tolerance phenotype of the diploid wheat (*T. monococcum*) was manifested by less effect on plant growth and a significant uptake of water producing maintenance of cell turgor. It seems that the tolerance of this cultivar was the consequence of its capacity to remove toxic Na^+ ions in the photosynthetic tissues. Moreover, this wheat cultivar induces the antioxidative enzymes CAT and SOD more efficiently than the other cultivar.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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