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Photosynthetic pigments, ionic and antioxidative behaviour of hulled tetraploid wheat in response to NaCl

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Abstract

Salt stress causes extensive losses to agricultural crops, including wheat, throughout the world and has been the focus of wide research. Though, information is scarce on the potential of ancient wheat relatives in tackling this major limiting factor. Thus, six hulled tetraploid wheat genotypes (HW) were compared to a free-threshing durum wheat genotype (FTW) under different NaCl concentrations, ranging from 0 to 150 mM, at early growth stages in a sand culture experiment. Salt stress induced significant declines in the leaf chlorophyll (Chl) *a*, Chl *b*, total Chl, and carotentoid contents; the extent of the declines was greater in FTW compared to HW. Mean leaf proline (3.6-fold) and Na^+ (1.58-fold) concentrations and Na^+/K^+ (2.48-fold) drastically increased with 150 mM of NaCl; the magnitude of the increases was greater in HW compared to FTW. While the carotenoids concentration decreased with progressive salinity both in HW and FTW, the activities of antioxidant enzymes, *i.e.*, catalase, ascorbate peroxidase, and peroxidase were reduced in FTW, but remained unchanged in HW. The above responses to 150 mM NaCl were associated with a significant decrease in shoot dry mass of FTW and lack of significant changes in that of HW. Findings of the present study could help pave the way for further studies on physiological and molecular mechanisms of salt tolerance in these durum wheat relatives.

Additional key words: primitive wheat; root dry mass; salinity.

Introduction

Salinity is one of major environmental constraints for crop plants that cause extensive losses throughout the world (Sairam *et al.* 2002, Munns and Tester 2008). This problem is more severe in arid and semiarid regions, *i.e.,* drought-stricken areas of the world. Saline lands will, perhaps, continue to expand in a global scale in coming decades (Ashraf and Harris 2004). Salinity leads to osmotic stress, due to lowering the soil water potential (Winicov 1998), *i.e.*, physiological drought. Salinity may result in rising accumulation of Na^+ and Cl^- and decrease in the uptake of ions, such as K^+, Ca^{2+}, and Mg^{2+}, *i.e.*, ion-excess effect of salinity, nutrient deficiency (due to a decrease in the plant's ability to obtain nutrients from the root medium) (Sairam *et al.* 2002), and reactive oxygen species (ROS)-induced oxidative stress (Munns and Tester 2008). The continuing salt accumulation in the soils brings about alterations in many physiological processes of plants (Genc *et al.* 2007). A decline in the Chl content, along with reduction in the photosynthetic rate and efficiency, have also been observed in salt-treated plants (Munns 2002). Therefore, a plant stricken by high salinity may experience reduced shoot and root growth, yield losses, and eventual death. In order to cope with the osmotic and ionic effects of salinity, some plant species have developed an array of up- and down-regulations in their physiological and biochemical processes, rendering them more tolerant to this stressor (Sairam *et al.* 2002). Some of these changes include osmotic adjustment by accumulation of compatible solutes, such as amino acids, polyols, and carbohydrates and regulatory mechanisms for ion transport (Ashraf and Harris 2004). Free proline is usually the most prevalent amino acid that is synthesized and accumulated in stressed plants of different species (Fougere *et al.* 1991).

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Abbreviations: APX – ascorbate peroxidase; Car – carotenoids; CAT – catalase; Chl – chlorophyll; Chl_{tot} – total chlorophyll; C0 – control; DTT – α-dithiothreitol; FM – fresh mass; FTW – free-threshing durum wheat genotype; HW – hulled tetraploid wheat group of genotypes; LSD – least significant difference; POX – peroxidase; PVP – polyvinyl pyrrolidone; ROS – reactive oxygen species; RDM – root dry mass; RH – relative humidity; S50 – 50 mM NaCl; S100 – 100 mM NaCl; S150 – 150 mM NaCl; SDM – shoot dry mass; SOD – superoxide dismutase; ST – salinity tolerance.

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Most crop plants belong to the group of glycophytes that are sensitive to salinity. Among cereals, bread wheat (*Triticum aestivum*, a hexaploid with genomes A, B, and D) is moderately salt tolerant. Durum wheat (*Triticum turgidum* ssp. Durum, a tetraploid with genomes A and B) is less tolerant (Munns and Tester 2008), presumably because it lacks the Na^+ excluding locus *Kna1* that is found on the D genome (Dvořák 1994).

Domestication and breeding programs during the centuries have strongly narrowed the genetic diversity in many crop species, rendering them more vulnerable to environmental stresses (Nevo 2004). There is a notable genetic diversity in wild or less-developed relatives of wheat, *i.e.*, they are potentially rich genetic resources of many valuable traits including salt and drought tolerance (Nevo and Chen 2010). Hulled wheats (also known as ancient or primitive wheats) are probably among the earliest domesticated plants (Nesbitt and Sammuel 1995) and have been used as staple crop for several centuries. These rather ancient wheats are found at all three levels of ploidy (*i.e.*, di-, tetra-, and hexaploid) and may be hired as resources of biotic and abiotic tolerance genes (Schmid *et al.* 1996) for modern wheats (also known as free-threshing wheats). One of the most efficient ways to increase the wheat yield in saline soils is to improve the salt tolerance of modern wheat genotypes; wild or semi-domesticated relatives might be potent to play a role in this regard. Although the mechanisms, by which salt affects plants, have been studied extensively, to our knowledge, little information is available on stress tolerance, in general, and physiological characterization in response to salinity, in particular, of the less-known hulled wheats (Ehsanzadeh *et al.* 2009). The present investigation was, therefore, undertaken to study the effect of NaCl on growth, photosynthetic pigments, ion balance, and some physiological traits in six hulled tetraploid wheat genotypes in comparison to a durum wheat cultivar at early growth stages.

Materials and methods

Plant material and growth conditions: The experiment was carried out in a growth chamber at the Isfahan University of Technology, Isfahan, Iran, from December 2012 to January 2013. Temperature, day length, relative humidity, and PAR were set at 20 (± 2ºC), 12 h, 65–75%, and 300 μmol(photon) m^{-2} s^{-1}, respectively. Seven tetraploid wheat genotypes including six HW accessions (*Triticum turgidum* subspp. *dicoccum*) and one FTW (*Triticum. turgidum* subspp. *durum*) cultivar were used in the experiment. The HW genotypes namely, 'Singerd', 'Zarneh', 'Joneghan', 'Khoygan', 'Shahrekord', and 'Ozonbelagh' had been collected from central Iran. The FTW genotype, 'Yavaroos', was obtained from Iran's Seed and Plant Improvement Institute, Karaj, Iran. Ten healthy seeds of the seven tetraploid wheat genotypes were sown into washed sand in plastic pots (10 × 10 × 20 cm). The sand culture was preferred to other forms of hydroponic culture, as roots are supported, the frequent subirrigation and drainage avoids hypoxia, and there is no breakage of lateral roots as it can occurs in unsupported hydroponics when the solution is changed (Miller 1987). From emergence until the two-leaf stage, seedlings were irrigated with half strength Hoagland's nutrient solution (Hoagland and Arnon 1950), prior the NaCl treatment. Five seedlings for each genotype were kept in each pot. The control plants (C0) were subjected to the nonsaline Hoagland's solution. NaCl (50 mM) concentration was applied in one step to the seedlings (S50), when they reached their three-leaf stage. In order to avoid osmotic shock, however, wheat seedlings of the higher salt concentrations were subjected to increasing incremental concentrations of 50 mM NaCl every two days until the final concentrations of 100 (S100) and 150 mM (S150) were achieved. Three weeks after the beginning of the NaCl treatment (*i.e.*, at seven-leaf stage), the plants were measured and harvested.

Chl and carotenoid (Car) concentrations: The Chl concentration was determined using fully expanded leaves. A fresh leaf sample of 0.1 g was ground and extracted with 5 ml of 80% (v/v) acetone in the dark. The slurry was filtered, centrifuged at 5,000 × *g* for 10 min and absorbance was determined at 645, 663, and 470 nm, for Chl *a*, Chl *b*, and Car concentrations, respectively, using a spectrophotometer (*U-1800 UV/VIS, Hitachi*, Japan). Concentrations of Chl *a*, Chl *b*, Chl_{tot}, and Car were determined according to Lichtenthaler and Wellburn (1994).

Root and shoot dry mass: Roots and shoots were separated, then dried at 70°C for 48 h to determine the root dry mass (RDM) and shoot dry mass (SDM).

Ion concentrations: The leaf samples were dried in an oven (70°C for 48 h), weighed and ground. The samples were ashed at 550°C for 4 h. Inorganic ions were extracted with 10 mL of 2 N hydrochloric acid (HCl), then the mixtures were heated at 90°C to remove hydrochloric acid and, subsequently, the volume was increased to 100 mL. The Na^+ and K^+ concentrations were measured using a flame photometer (*Corning Flame Photometer 410, Corning Medical and Scientific*, Halstead Essex, UK). Concentrations of K^+ and Na^+ ions were estimated by referring to the curve prepared with known concentrations of the ions.

Proline concentration: The free proline concentration was determined according to Bates *et al.* (1973). Leaf samples of 100 mg of fresh mass (FM) was grinded in 0.5 mL of 3% aqueoussulfosalicylic acid. The mixture was centrifuged (*5810R, Eppendorf Refrigerated Centrifuge*, Germany) at 10,000 × *g* for 5 min at room temperature. For preparing the reaction mixture, 0.1 mL of the supernatant was added to 0.1 mL of 3% sulfosalicylic acid, 0.2 mL of glacial acetic acid, and 0.2 mL of acetic ninhydrin in a separate tube. The reaction mixture was boiled in a water bath at 96°C for 60 min. After cooling the mixture on ice, 1 mL of toluene was added and thoroughly mixed; the toluene phase was separated after 5 min and its absorbance was measured at 520 nm spectrophotometrically ((*U-1800 UV/VIS, Hitachi*, Japan). The concentration of proline was calculated using the standard curve prepared with known concentrations of proline.

Assays for antioxidant enzyme activities

Enzyme extraction: Leaf samples (0.1 g FM) were ground in a prechilled mortar and pestle and homogenized with 1 mL of 50 mM Na-phosphate buffer (pH 7), containing 2 mM α-dithiothreitol (DTT), 2 mM EDTA, 0.2% Triton X-100, 50 mM Tris-HCl, and 2% polyvinyl pyrrolidone (PVP). Each homogenate was transferred to a tube and centrifuged at 4°C for 15 min at 15,000 × *g*. All steps of the extraction were carried out at 4°C. The supernatant was used to assay the following antioxidant enzyme activity and the protein content.

Enzyme assays: Catalase (CAT, EC 1.11.1.6) activity was determined by measuring the conversion rate of hydrogen peroxide to water and oxygen molecules. For enzyme assay, the reaction medium (3 mL) contained 2,950 µl of the 50 mM Na-phosphate buffer (pH 7.0), containing 4.50 µl of H_2O_2 (30%) and 50 µl of the enzyme extract. The decrease in absorbance at 240 nm was monitored for 1 min spectrophotometrically (*U-1800 UV/VIS, Hitachi*, Japan). CAT (extinction coefficient = 39.4 mM^{-1} cm^{-1}) activity was expressed as unit per mg of protein (Chance and Maehly 1955). One unit of CAT activity is defined as the amount of CAT necessary to decompose 1.0 µM of H_2O_2 per min.

Ascorbate peroxidase (APX, EC 1.11.1.11) activity was determined by measuring the oxidation of ascorbate to dehydroascorbate as described by Nakano and Asada (1981). The reaction medium (3 mL) for APX activity assay contained 2,845 µl of the 50 mM Na-phosphate buffer (pH 7.0), containing 4.50 µl of H_2O_2 (30%), 100 µl of 5 mM ascorbate, and 50 µl of the enzyme extract. The decrease in absorbance at 290 nm was monitored for 2 min spectrophotometrically (*U-1800 UV/VIS, Hitachi*, Japan). APX (extinction coefficient = 2.8 mM^{-1} cm^{-1}) activity was expressed as unit per mg of protein. One unit of APX activity was defined as the amount of enzyme needed for oxidation of 1 µmol of ascorbate per min.

Peroxidase (POX, EC 1.11.1.7) activity was determined with guaiacol. The assay mixture (3 mL) contained 2,943 mL of the 50 mM Na-phosphate buffer (pH 7.0), containing 4.50 µl of H_2O_2 (30%), 3.35 µl of guaiacol, and 50µl of enzyme extract. The increase in absorbance at 470 nm, due to degradation of H_2O_2, was monitored for 2 min spectrophotometrically (*U-1800 UV/VIS, Hitachi*, Japan). POX (extinction coefficient = 26.61 mM^{-1} cm^{-1}) activity was expressed as unit per milligram of protein (Herzog and Fahimi 1973). One unit of POX activity represents the amount of enzyme that catalyses the oxidation of 1.0 µM of guaiacol in 1 min.

The total protein content of leaf samples was determined using the method described by Bradford (1976). Bradford dye reagent contained 50 mL of ethanol (95%), 100 mL of orthophosphoric acid, and 100 mg of Coomassie Brilliant Blue diluted to make up 1,000 mL. A total of 2,992 µl of the Bradford reagent was added to test tubes containing 6 µl of the protein extract. The tubes containing the extract and Bradford reagent were subsequently incubated at room temperature for 30 min, thoroughly mixed, and the absorbance was measured at 595 nm (*U-1800 UV/VIS, Hitachi*, Japan) and compared to bovine serum albumin (BSA) as a standard.

Experimental design and statistical analysis: A factorial experiment (NaCl at four concentrations and seven genotypes) was conducted using a randomized complete block design with three replications. Analysis of variance (*ANOVA*) was carried out using *Statistical Analysis Software version 8.2* (*SAS Institute Inc.*, Cary, North Carolina, USA). Orthogonal independent comparisons were conducted for differences within and between the two types of tetraploid wheat, *i.e.*, HW *vs*. FTW and for their interaction with the salt treatment. The mean comparisons were conducted using *Fisher*'s least significant difference (LSD) at $p \leq 0.050$.

Results

Analyses of variances indicated that all measured traits were significantly affected by salt, except for RDM and Chl *a/b* (Table 1). Furthermore, genotypes were found significantly different in most of the measured traits with the exception of Chl *a*, Chl_{tot}, Chl *a/b,* Car, and proline concentrations. *ANOVA* showed that HW differed significantly from FTW in all measured attributes, except for SDM, Chl *a*, Chl_{tot}, Car, proline, and K^+ concentrations. However, statistically significant differences were detected within HW only for SDM, CAT and POX activities, and K^+ concentration. Interacting effects of salinity × genotype, salinity × genotype, and salinity × HW were found to be statistically significant for all measured traits, albeit with the exception of Chl *a/b*.

Table 1. Analysis of variances (mean squares) for root dry mass (RDM), shoot dry mass (SDM), chlorophyll (Chl) *a,* Chl *b,* Chl$_{tot}$, Chl *a/b*, carotenoids (Car), proline concentration, catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POX) activities, Na^+ and K^+ concentrations, and Na^+/K^+ ratio of seven tetraploid wheat genotypes, grouped in two types of six hulled wheat accessions and a free-threshing wheat genotype under four concentrations of NaCl in three replications. G – tetraploid wheat genotypes; HW – hulled wheat accessions; FTW – free-threshing wheat genotype; S – NaCl concentration; *R* – replication;. df – degrees of freedom; ns – not significant; Error – within group variance; * – $P \leq 0.05$; ** – $P \leq 0.01$.

Trait	Sources of variations								
	R	S	G	HW *vs*. FTW	HW	S × G	S × Group	S × HW	Error
df	2	3	6	1	5	18	3	15	54
RDM ($\times 10^{-4}$)	0.3^{ns}	1.0^{ns}	0.2^{*}	0.9^{**}	2.4^{ns}	1.0^{ns}	1.7^{ns}	1.6^{ns}	1.0
SDM ($\times 10^{-4}$)	0.5^{ns}	14.5^{**}	12.2^{**}	0.2^{ns}	8.0^{**}	5.8^{**}	15.0^{**}	6.0^{**}	2.0
Chl *a*	0.002^{ns}	0.352^{**}	0.014^{ns}	0.0003^{ns}	0.016^{ns}	0.015^{**}	0.156^{**}	0.049^{**}	0.006
Chl *b*	0.001^{ns}	0.051^{**}	0.002^{*}	0.008^{**}	0.002^{ns}	0.002^{**}	0.022^{**}	0.007^{**}	0.001
Chl *a/b*	0.220^{ns}	0.683^{ns}	0.509^{ns}	1.860^{**}	0.529^{ns}	0.363^{ns}	0.418^{ns}	0.478^{ns}	0.28
Chl *tot*	0.003^{ns}	0.623^{**}	0.014^{ns}	0.016^{ns}	0.017^{ns}	0.027^{**}	0.277^{**}	0.084^{**}	0.008
Car	1.216^{ns}	31.513^{**}	3.053^{ns}	0.00004^{ns}	3.510^{ns}	8.560^{**}	15.330^{**}	9.998^{**}	3.34
Proline	0.019^{ns}	19.913^{**}	0.218^{ns}	0.320^{ns}	0.155^{ns}	0.275^{**}	8.753^{**}	2.460^{**}	0.122
CAT	0.042^{ns}	0.228^{**}	0.486^{**}	0.264^{**}	0.098^{*}	0.261^{**}	0.896^{**}	0.103^{**}	0.041
APX	0.001^{ns}	0.017^{**}	0.020^{**}	0.003^{**}	0.003^{ns}	0.011^{**}	0.037^{**}	0.005^{**}	0.001
POX	2.381^{ns}	91.848^{**}	82.648^{**}	32.964^{**}	16.973^{*}	41.242^{**}	174.441^{**}	14.998^{**}	5.322
Na^+	0.013^{ns}	4.511^{**}	$0{\cdot}189^{**}$	0.089^{**}	0.077^{ns}	0.076^{**}	2.111^{**}	0.622^{**}	0.029
K^+	0.001^{ns}	0.240^{**}	0.045^{**}	0.066^{ns}	0.053^{**}	0.023^{**}	0.111^{**}	0.051^{**}	0.004
Na^+/K^+	0.019^{ns}	17.050^{**}	0.656^{**}	0.101^{**}	0.334^{ns}	0.197^{*}	7.701^{**}	2.258^{**}	0.083

Mean RDM did not significantly change with the salt concentration and it was in the range of 0.011 to 0.021 g per plant, however, mean SDM varied from 0.045 to 0.075 g per plant for the genotypes examined in this study (Table 2). Mean leaf Chl *a*, Chl *b*, Chl$_{tot}$, and Car concentrations decreased by 43, 44, 42, and 30%, respectively, when the tetraploid wheat genotypes were grown at the presence of S150, relative to control (C0) conditions. Mean Chl *a/b* of the tetraploid wheat genotypes tended to increase (13%) when grown under S150. Ranges of mean pigment concentrations for the examined genotypes on a mg g^{-1} basis were 0.5 to 0.61 for Chl *a*, 0.18 to 0.21 for Chl *b*, 0.69 to 0.79 for Chl$_{tot}$, and 5.98 to 7.38 for Car. Mean Chl *a/b* of the genotypes ranged from 2.66 to 3.19.

Mean leaf proline and Na^+ concentrations and Na^+/K^+ of plants increased by 3.6-, 1.58-, and 2.48-fold, respectively, when subjected to S150, compared to C0 (Table 2). The mean leaf K^+ concentration decreased by 28% under S150, relative to C0 (Table 2). Mean osmolyte concentrations of the genotypes were from 1.43 to 1.78 µmol g^{-1} for proline, 1.028 to 1.433 mmol g^{-1} for Na^+, and 0.567 to 0.745 mmol g^{-1} for K^+. Mean Na^+/K^+ of the genotypes ranged from 1.593 to 2.265. Mean CAT, APX, and POX activities declined by 50, 60, and 71%, respectively, with an increase in the NaCl concentration of the medium from 0 to 150 mM (Table 2). Ranges of mean antioxidative enzymes activities for the genotypes, expressed as Unit mg^{-1} (protein), were from 0.109 to 0.695 for CAT, 0.025 to 0.148 for APX, and 1.370 to 8.931 for POX.

Leaf Chl *a*, Chl *b*, and Chl$_{tot}$ concentrations of all tetraploid wheat genotypes decreased with the increase in NaCl concentration, though the extent of the decreases varied with the genotype (Table 3). The greatest and smallest reductions in the Chl *a* concentration were observed in the genotypes 'Zarneh' (60%), 'Yavaroos' (53%), and 'Singerd' (15%). The greatest and smallest decreases in Chl *b* were observed in the genotypes 'Zarneh' (57%) and 'Shahrekord' (32%) and those of Chl$_{tot}$ were observed in 'Zarneh' (60%), 'Yavaroos' (53%), and 'Singerd' (23%). The Car concentration in leaves responded to the NaCl concentration in a genotype-dependent manner (Table 3). The Car concentration of the genotype 'Singerd' did not vary notably, while that of 'Yavaroos' decreased significantly with NaCl concentration and those of other genotypes increased, when treated with S50 and/or S100, and decreased, when subjected to S100 and/or S150.

The leaf proline concentration of all durum wheat genotypes increased drastically when the plants were grown under S100 and S150 (Table 3). The magnitude of the increase for the genotype 'Singerd' (2.5-fold) was found to be smaller compared to the increases found in other genotypes (*i.e.*, 3.6- to 6.3-fold). The leaf Na^+ concentration of all tetraploid wheat genotypes drastically increased when they were subjected to NaCl, though the magnitude of the increase varied in different genotypes (Table 3). The greatest and smallest increases were detected in the genotypes 'Singerd' (263%), 'Joneghan' (276%), and 'Yavaroos' (97%). The leaf K^+ concentration was affected by NaCl in a genotype-dependent manner (Table 3). The leaf K^+ concentration in the genotypes 'Singerd' and 'Joneghan' did not change significantly, but it decreased significantly in the remaining genotypes. The Na^+/K^+ ratio for all tetraploid wheat genotypes increased several fold with the NaCl concentration, albeit the magnitude of the increases differed with the genotype

Table 2. Mean comparisons for root dry mass (RDM), shoot dry mass (SDM), chlorophyll (Chl) *a,* Chl *b,* Chl_{tot}, Chl *a/b*, carotenoids (Car), and proline concentration, catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POX) activities, Na^+ and K^+ concentrations and Na^+/K^+ ratio of seven tetraploid wheat genotypes at four concentrations of NaCl. ST – salinity tolerance. Data are the mean ($n = 21$ for NaCl; $n = 12$ for genotype). Values within a group in a row bearing *different superscripts* are significantly different at $P \leq 0.05$. LSD – least significant difference at $P \leq 0.05$. Each unit of specific activities of enzymes is said to be the amount of enzyme necessary to convert 1.0 µM of H_2O_2 per min (*i.e.* for CAT), to oxidize 1 µmol of ascorbate per min (*i.e.* for APX), and to oxidize 1.0 µM of guaiacol per min (*i.e.* for POX).

Trait	NaCl concentration [mM]					Genotype							
	0	50	100	150	LSD	Singerd	Joneghan	Khoygan	Shahrekord	Zarneh	Ozonbelagh	Yavaroos	LSD
RDM [g $plant^{-1}$]	0.014^{a}	0.019^{a}	0.015^{a}	0.013^{a}	0.009	0.014^{ab}	0.012^{ab}	0.014^{ab}	0.013^{ab}	0.024^{a}	0.011^{b}	0.021^{ab}	0.011
SDM [g $plant^{-1}$]	0.063^{ab}	0.050^{c}	0.07^{a}	0.060^{b}	0.01	0.062^{bc}	0.051^{cd}	0.063^{bc}	0.069^{ab}	0.062^{bc}	0.045^{d}	0.075^{a}	0.017
Chl *a* [mg g^{-1}(FM)]	0.69^{a}	0.63^{a}	0.55^{b}	0.39^{c}	0.07	0.50^{a}	0.61^{a}	0.59^{a}	0.55^{a}	0.57^{a}	0.55^{a}	0.58^{a}	0.09
Chl *b* [mg g^{-1}(FM)]	0.23^{a}	0.22^{a}	0.18^{b}	0.13^{c}	0.02	0.18^{b}	0.18^{b}	0.19^{ab}	0.18^{b}	0.21^{a}	0.18^{ab}	0.18^{b}	0.03
Chl *a/b*	2.87^{a}	2.87^{a}	3.13^{a}	3.23^{a}	0.44	2.83^{a}	3.13^{a}	3.14^{a}	3.20^{a}	2.66^{a}	3.01^{a}	3.19^{a}	0.58
Chl_{tot} [mg g^{-1}(FM)]	0.91^{a}	0.85^{a}	0.73^{b}	0.52^{c}	0.08	0.69^{a}	0.76^{a}	0.78^{a}	0.74^{a}	0.79^{a}	0.74^{a}	0.76^{a}	0.10
Car [mg g^{-1}(FM)]	7.65^{ab}	8.13^{a}	6.53^{bc}	5.39^{c}	1.51	5.98^{a}	6.53^{a}	7.30^{a}	7.38^{a}	6.92^{a}	7.19^{a}	7.16^{a}	1.99
Proline [µmol g^{-1}(FM)]	0.57^{d}	1.05^{c}	2.25^{b}	2.63^{a}	0.29	1.45^{a}	1.71^{a}	1.63^{a}	1.72^{a}	1.78^{a}	1.66^{a}	1.43^{a}	0.38
CAT [Unit mg^{-1}(protein)]	0.434^{a}	0.209^{b}	0.241^{b}	0.233^{b}	0.17	0.329^{b}	0.246^{b}	0.288^{b}	0.141^{b}	0.143^{b}	0.109^{b}	0.695^{a}	0.22
APX [Unit mg^{-1}(protein)]	0.102^{a}	0.064^{b}	0.042^{b}	0.044^{b}	0.03	0.061^{b}	0.064^{b}	0.062^{b}	0.048^{b}	0.032^{b}	0.025^{b}	0.148^{a}	0.04
POX [Unit mg^{-1}(protein)]	6.548^{a}	3.203^{b}	2.386^{b}	1.917^{b}	1.90	3.762^{bc}	4.372^{b}	2.374^{bc}	1.979^{bc}	1.804^{c}	1.370^{c}	8.931^{a}	2.51
Na^+ [mmol g^{-1}(DM)]	0.628^{c}	1.198^{b}	1.592^{a}	1.623^{a}	0.14	1.243^{b}	1.222^{b}	1.310^{ab}	1.339^{ab}	1.433^{a}	1.241^{b}	1.028^{c}	0.19
K^+ [mmol g^{-1}(DM)]	0.829^{a}	0.671^{b}	0.608^{c}	0.598^{c}	0.06	0.680^{ab}	0.567^{c}	0.631^{bc}	0.745^{a}	0.675^{ab}	0.737^{a}	0.697^{ab}	0.07
Na^+/K^+	0.792^{c}	1.817^{b}	2.648^{a}	2.725^{a}	0.24	1.886^{bc}	2.173^{ab}	2.195^{ab}	1.910^{b}	2.265^{a}	1.943^{b}	1.593^{c}	0.31

Table 3. Mean comparisons for the effects of interaction of genotype × salinity on shoot dry mas (SDM), leaf chlorophyll (Chl) *a*, Chl *b*, Chl_{tot}, carotenoids (Car), and proline concentrations, catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POX) antioxidative enzymes activities, Na^+ and K^+ concentrations and Na^+/K^+ ratio.. Data are the mean ($n = 3$). Values in a column bearing *different superscripts* are significantly different at $P \leq 0.05$. LSD – least significant difference at $P \leq 0.05$.

NaCl [mM]	Genotype	SDM [g per plant]	Chl *a* [mg g^{-1}(FM)]	Chl *b*	Chl_{tot}	Car	Proline [µmol g^{-1}(FM)]	CAT [Unit mg^{-1}(protein)]	APX	POX	Na^+ [mmol g^{-1}(DM)]	K^+	Na^+/K^+
0	Singerd	0.052^{a-f}	0.54^{c-i}	0.22^{a-f}	0.77^{c-i}	6.48^{a-d}	0.65^{g-j}	0.74^{bc}	0.142^{cd}	9.40^{b}	0.51^{h}	0.76^{c-e}	0.66^{k}
	Joneghan	0.059^{a-f}	0.70^{a-d}	0.22^{b-e}	0.78^{c-i}	5.01^{bcd}	0.44^{j}	0.48^{b-e}	0.161^{bc}	9.27^{b}	0.48^{h}	0.60^{g-l}	0.80^{jk}
	Khoygan	0.073^{a-f}	0.70^{abc}	0.24^{abc}	0.95^{abc}	8.19^{abc}	0.54^{ij}	0.08^{e}	0.019^{fg}	2.31^{c}	0.57^{h}	0.78^{b-e}	0.73^{k}
	Shahrekord	0.060^{a-f}	0.64^{a-h}	0.23^{a-f}	0.86^{a-e}	8.15^{abc}	0.54^{ij}	0.09^{e}	0.034^{efg}	1.52^{c}	0.67^{fgh}	0.92^{ab}	0.73^{k}
	Zarneh	0.072^{a-f}	0.78^{a}	0.28^{a}	1.06^{a}	9.87^{a}	0.53^{ij}	0.12^{e}	0.033^{efg}	1.23^{c}	0.97^{efg}	0.82^{bcd}	1.38^{ij}
	Ozonbelagh	0.036^{efg}	0.72^{ab}	0.22^{b-e}	0.94^{abc}	6.43^{a-d}	0.67^{g-j}	0.05^{e}	0.037^{efg}	0.59^{c}	0.64^{gh}	1.03^{a}	0.62^{k}
	Yavaroos	0.087^{abc}	0.77^{ab}	0.25^{ab}	1.03^{ab}	9.46^{a}	0.68^{g-j}	1.48^{a}	0.288^{a}	21.53^{a}	0.56^{h}	0.90^{abc}	0.62^{k}
50	Singerd	0.053^{c-g}	0.51^{e-j}	0.19^{c-h}	0.70^{d-j}	6.18^{a-d}	1.22^{d-i}	0.06^{e}	0.012^{g}	1.42^{c}	1.04^{def}	0.69^{d-i}	1.51^{hi}
	Joneghan	0.020^{g}	0.72^{ab}	0.22^{b-e}	0.94^{bc}	8.69^{a-b}	1.34^{d-h}	0.12^{e}	0.053^{efg}	2.48^{c}	1.11^{de}	0.56^{h-l}	1.99^{f-i}
	Khoygan	0.041^{fg}	0.67^{a-f}	0.26^{ab}	0.92^{abc}	9.85^{a}	1.39^{d-g}	0.06^{e}	0.024^{fg}	1.71^{c}	1.29^{b-e}	0.54^{i-l}	2.38^{c-g}
	Shahrekord	0.062^{a-f}	0.62^{a-h}	0.23^{a-f}	0.84^{b-f}	9.54^{a}	0.96^{f-j}	0.10^{e}	0.088^{c-g}	3.09^{c}	1.38^{bcd}	0.75^{def}	1.83^{hig}
	Zarneh	0.053^{d-g}	0.61^{a-h}	0.24^{a-d}	0.85^{b-f}	5.13^{bcd}	1.05^{f-i}	0.10^{e}	0.037^{efg}	1.97^{c}	1.36^{bcd}	0.66^{e-k}	2.05^{e-h}
	Ozonbelagh	0.044^{efg}	0.62^{a-h}	0.22^{b-e}	0.84^{b-f}	9.39^{a}	0.84^{g-j}	0.10^{e}	0.013^{g}	1.78^{c}	1.06^{de}	0.75^{def}	1.42^{ij}
	Yavaroos	0.078^{a-e}	0.68^{a-e}	$0.21^{b-g\backslash}$	0.89^{a-d}	8.15^{abc}	0.58^{hij}	0.92^{b}	0.224^{ab}	9.97^{b}	1.13^{cde}	0.74^{d-g}	1.53^{hi}
100	Singerd	0.089^{a}	0.51^{e-j}	0.19^{c-h}	0.71^{d-j}	5.21^{bcd}	1.69^{c-f}	0.11^{e}	0.027^{fg}	0.68^{c}	1.57^{ab}	0.62^{f-k}	2.54^{b-f}
	Joneghan	0.060^{a-f}	0.63^{a-h}	0.18^{d-i}	0.82^{c-h}	7.54^{a-d}	2.33^{abc}	0.23^{de}	0.017^{fg}	2.87^{c}	1.51^{ab}	0.52^{kl}	2.89^{abc}
	Khoygan	0.069^{a-f}	0.66^{a-g}	0.18^{e-j}	0.83^{b-g}	6.90^{a-d}	1.88^{bcd}	0.64^{bcd}	0.109^{cde}	1.76^{c}	1.78^{a}	0.68^{d-i}	2.63^{a-e}
	Shahrekord	0.070^{a-f}	0.50^{f-j}	0.13^{i-l}	0.64^{g-l}	4.93^{bcd}	2.72^{a}	0.19^{e}	0.039^{efg}	2.72^{c}	1.65^{ab}	0.67^{e-i}	2.48^{b-f}
	Zarneh	0.062^{a-f}	0.60^{b-h}	0.23^{a-e}	0.83^{b-h}	8.82^{ab}	2.62^{ab}	0.13^{e}	0.036^{efg}	1.91^{c}	1.80^{a}	0.67^{e}-j	2.69^{a-d}
	Ozonbelagh	0.054^{b-g}	0.48^{g-k}	0.17^{f-k}	0.65^{f-l}	6.32^{a-d}	2.74^{abc}	0.17^{e}	0.023^{fg}	1.51^{c}	1.49^{abc}	0.47^{l}	3.17^{a}
	Yavaroos	0.088^{ab}	0.52^{d-j}	0.16^{i-l}	0.68^{e-k}	6.00^{a-d}	1.81^{cde}	0.22^{de}	0.042^{efg}	2.00^{c}	1.33^{b-e}	0.62^{f-k}	2.13^{d-h}
150	Singerd	0.052^{d-g}	0.46^{ijk}	0.13^{i-l}	0.59^{i-m}	6.05^{a-d}	2.26^{abc}	0.40^{cde}	0.065^{d-g}	3.55^{c}	1.85^{a}	0.65^{e-k}	2.83^{abc}
	Joneghan	0.065^{a-f}	0.39^{ijk}	0.12^{i-l}	0.52^{j-m}	4.91^{bcd}	2.75^{a}	0.16^{e}	0.023^{fg}	2.87^{c}	1.79^{a}	0.60^{g-l}	3.01^{abc}
	Khoygan	0.067^{a-f}	0.34^{jk}	0.11^{k}	0.45^{lm}	4.27^{cd}	2.74^{a}	0.38^{cde}	0.095^{c-f}	3.74^{c}	1.59^{ab}	0.52^{jkl}	3.04^{ab}
	Shahrekord	0.085^{a-d}	0.47^{ijk}	0.15^{g-l}	0.63^{h-m}	6.93^{a-d}	2.68^{a}	0.19^{e}	0.032^{efg}	0.59^{c}	1.65^{b}	0.64^{e-k}	2.60^{a-f}
	Zarneh	0.061^{a-f}	0.31^{k}	0.12^{kl}	0.43^{m}	3.89^{d}	2.93^{a}	0.23^{de}	0.023^{fg}	2.12^{c}	1.61^{ab}	0.55^{i-l}	2.94^{abc}
	Ozonbelagh	0.046^{efg}	0.41^{ijk}	0.15^{h-l}	0.56^{j-m}	6.64^{a-d}	2.43^{a}	0.11^{e}	0.026^{fg}	1.60^{c}	1.72^{a}	0.70^{d-h}	2.56^{a-f}
	Yavaroos	0.045^{a-e}	0.36^{ijk}	0.121^{jkl}	0.49^{klm}	5.04^{bcd}	2.67^{a}	0.17^{e}	0.040^{efg}	2.23^{c}	1.10^{de}	0.52^{jkl}	2.095^{d-h}
LSD		0.03	0.18	0.55	0.20	3.99	0.76	0.44	0.08	5.03	0.38	0.15	0.63

Table 4. Mean comparisons for the interaction effects of tetraploid wheat type × salinity on shoot dry mass (SDM), chlorophyll (Chl) *a*, Chl *b*, Chl$_{tot}$, carotenoids (Car), and proline concentrations, catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POX) activities, Na^+ and K^+ concentrations, and Na^+/K^+ ratio. Data are the means ($n = 3$). Values in a row with *different superscripts* are significantly different at $P \leq 0.05$. LSD – least significant difference at $P \leq 0.05$.

Parameters	HW NaCl [mM] 0	 50	 100	 150	FTW NaCl [mM] 0	 50	 100	 150	 LSD
RDM [g plant^{-1}]	0.012^{a}	0.018^{a}	0.015^{a}	0.013^{a}	0.028^{a}	0.025^{a}	0.018^{a}	0.015^{a}	0.019
SDM [g plant^{-1}]	0.056^{bc}	0.045^{c}	0.067^{ab}	0.063^{ab}	0.087^{a}	0.078^{ab}	0.088^{a}	0.045^{c}	0.029
Chl *a* [mg g^{-1}(FM)]	0.679^{ab}	0.624^{bc}	0.562^{c}	0.398^{d}	0.774^{a}	0.683^{ab}	0.519^{cd}	0.364^{d}	0.158
Chl *b* [mg g^{-1}(FM)]	0.234^{ab}	0.224^{ab}	0.181^{c}	0.128^{d}	0.254^{a}	0.206^{bc}	0.155^{cd}	0.121^{d}	0.050
Chl *tot* [mg g^{-1}(FM)	0.891^{ab}	0.849^{bc}	0.7445^{c}	0.528^{d}	1.028^{a}	0.889^{abc}	0.675^{cd}	0.485^{d}	0.189
Car [mg g^{-1}(FM)]	7.350^{ab}	8.130^{ab}	6.620^{ab}	5.447^{b}	9.455^{a}	8.150^{ab}	6.001^{ab}	5.042^{b}	3.535
Proline [µmol g^{-1}(FM)]	0.560^{d}	1.134^{c}	2.329^{ab}	2.632^{a}	0.681^{cd}	0.583^{cd}	1.806^{b}	2.672^{a}	0.646
CAT [Unit mg^{-1}(protein)]	0.260^{c}	0.208^{c}	0.240^{c}	0.244^{c}	1.477^{a}	0.917^{b}	0.220^{c}	0.165^{c}	0.395
APX [Unit mg^{-1}(protein)]	0.071^{b}	0.064^{b}	0.042^{b}	0.044^{b}	0.288^{a}	0.224^{a}	0.042^{b}	0.040^{b}	0.080
POX [Unit mg^{-1}(protein)]	4.051^{c}	3.202^{c}	1.917^{c}	2.412^{c}	21.525^{a}	9.970^{b}	2.00^{c}	2.228^{c}	4.616
Na^+ [mg g^{-1}(DM)]	0.640^{d}	1.197^{c}	1.591^{ab}	1.710^{a}	0.558^{d}	1.131^{c}	1.327^{bc}	1.098^{c}	0.325
K^+ [mg g^{-1}(DM)]	0.817^{a}	0.671^{b}	0.607^{b}	0.609^{b}	0.901^{a}	0.740^{ab}	0.623^{b}	0.524^{b}	0.180
Na^+/K^+	0.821^{c}	1.817^{b}	2.647^{a}	2.830^{a}	0.619^{c}	1.529^{b}	2.128^{b}	2.095^{b}	0.583

(Table 3). The greatest increases were observed in the genotypes ‘Khoygan’ (4.2-fold), ‘Joneghan’ (3.8-fold), and ‘Singerd’ (4.5-fold), while the smallest increases were observed in the genotypes ‘Yavaroos’ (3.4-fold) and ‘Zarneh’ (2.13-fold). CAT, APX, and POX activities of tetraploid wheats were affected by the NaCl concentration in a genotype-dependent manner (Table 3). While CAT and APX activities in the genotype ‘Yavaroos’ were the greatest at the C0 and S50, the genotype ‘Khoygan’ appeared to have the greatest antioxidant enzyme activities at S100 and S150, while ‘Singerd’ had the greatest CAT activity at S150 mM and APX activity at C0. The genotype ‘Yavaroos’ showed the greatest APX activity when grown at S50. The POX activity of the genotypes ‘Shahrekord’, ‘Zarneh’, ‘Ozonbelagh’, and ‘Yavaroos’ was not altered due to NaCl. The activity of POX in the genotypes ‘Yavaroos’, ‘Singerd’, and ‘Joneghan’ drastically declined when grown at the presence of NaCl concentrations above S50. SDM of HW remained unchanged, but that of FTW was reduced substantially (*i.e.*, 48%) when subjected to S150 compared with C0 (Table 4). Leaf Chl *a*, Chl *b*, and Chl$_{tot}$ concentrations of HW and FTW tended to decline with the increase in the NaCl concentration, but the extent of the decreases in HW was smaller relative to FTW (Table 4). Leaf proline concentrations of HW and FTW were enhanced, more or less, in proportion to increases in the Na^+ concentration and the Na^+/K^+ratio from C0 to S150 (Table 4), though the increases tended to be greater in HW than those in FTW. The leaf K^+ concentration of both HW and FTW were reduced with the increase in the NaCl concentration, albeit the decrease was more pronounced in FTW (Table 4). CAT, APX, and POX activities of HW were not significantly affected by NaCl, though that of FTW was drastically reduced when subjected to S100 and S150 compared with C0.

Discussion

It is obvious (Table 3 and 4) that both HW and FTW shared, more or less, the same decreasing trend in the leaf Chl concentration when grown under saline conditions, albeit the decrease in FTW appeared to be greater. The decrease in the leaf Chl concentration is reported to be due to increased chlorophyllase activity and Chl degradation and a disturbance in the biosynthesis of photosynthetic pigments (Singh and Dubey 1995). Reduction in the Chl concentration under saline conditions in hexaploid (Kiani-Pouya and Rasouli 2014) and tetraploid wheat (Ehsanzadeh *et al.* 2009) has been reported. Two somewhat opposing scenarios could be proposed for the stress-induced Chl destruction in tetraploid wheats, in general, and in FTW, in particular. In one scenario, the decrease in the Chl concentration might be seen as an adaptive strategy to avoid oxidative stress by reducing the amount of light intercepted and therefore reducing the amount of ROS generated by chloroplasts. In another scenario, overproduction of chloroplast-associated ROS during exposure of plants to salt stress might lead to an array of harmful effects including photosynthetic pigment destruction. Unlike the decrease in the Chl concentration, the Chl *a/b* ratio of the genotypes used in this study increased under saline conditions. During the process of Chl degradation, Chl *b* is converted into Chl *a* (Azizpour *et al.* 2010) and this might explain the increased Chl *a/b* ratio of plants subjected to S150 in our study. The decrease in the leaf

Chl concentration of the tetraploid wheat genotypes could be an important factor contributing to potential alterations in their photosynthetic activity. Photosynthetic activity of the tetraploid wheats was not measured herein. But it is reasonable to imagine that photosynthetic attributes have been adversely affected as a consequence of salt stress and that this adverse effect has been more substantial in the FTW.

Osmotically driven removal of water from cells may happen as a result of high Na^+ concentrations in the leaf apoplast (Parvaiz and Satyawati 2008). Increase in accumulation of specific ions, such as Na^+ and $Cl^–$, leads to ion-specific effects of salinity. Ionic toxicity of Na^+ is largely related to its competition with K^+ for binding sites crucial to metabolic processes that Na^+ cannot fulfill correctly (Bhandal and Malik 1988). Appropriate amounts of both K^+ and Ca^{2+} are needed to maintain the integrity of cell membranes and normal functioning of all metabolic processes (Debez *et al.* 2004). An elevated Na^+ concentration has been reported to decrease the uptake of K^+ and Ca^{2+} in different plant species (Gorham *et al.* 1987). Salt injury develops due to an inability of the cells to compartmentalize an excess of toxic ions, such as Na^+ or $Cl^–$, in the vacuole. Consequently, ions may build up in the cytoplasm and cell wall and inhibit enzyme activities and facilitate the cellular dehydration therein, respectively (Munns 2002). When the concentration of Na^+ in the root zone and its uptake by the plant increases, the uptake of K^+ is in turn decreased and the Na^+/K^+ ratio in plant tissue increases (Gorham *et al.* 1987). The ability to maintain the K^+ uptake and a low Na^+/K^+ ratio under salt stress can be an important mechanism of salt tolerance (Chen *et al.* 2007). Small Na^+/K^+ and Na^+/Ca^{2+} ratios are essential for normal cellular functions under saline conditions, and these ratios have been repeatedly shown as reliable parameters for salt tolerance estimation (Sairam *et al.* 2002). The high Na^+ concentration or Na^+/K^+ ratio may interfere with a number of enzymatic processes in the cytoplasm. The maintenance of enough K^+ in plant tissue under salinity seems to be related to the selective K^+ uptake, the appropriate cellular K^+ and Na^+ compartmentation, and distribution in the shoots (Munns 2002).

The HW genotypes showed the greater enhancement in their Na^+ (Table 4) and smaller decreases in K^+ (Table 4) concentrations with progressing salinity, compared to FTW. The Na^+/K^+ ratio increased in all genotypes with the progressive salinity and this ratio was invariably greater for HW compared to FTW under all concentrations of NaCl examined here (Table 4). From our data, it could be deduced that Na^+ exclusion capacity was smaller in HW than that of FTW. It may appear surprising that an association between the presumed Na^+ exclusion and salt tolerance in studied tetraploid wheat genotypes was lacking. Genotypic differences in Na^+ exclusion has been reported in other studies (Schachtman *et al.* 1991), but based on these studies, the interrelationships of salt exclusion capacity and salt tolerance are not consistent. Munns and James (2003) found a positive correlation between Na^+ exclusion and salt tolerance in *T. durum* ssp. *durum* selections, but did not observe such correlations in other tetraploid selections. Some studies have also reported positive correlations between salt tolerance and Na^+ exclusion in *T. aestivum* (Ashraf and O'Leary 1996, Poustini and Siosemardeh 2004), but some studies have not found any correlations (Ashraf and McNeilly 1998) or some other found only weak associations (Huang *et al.* 2006). Findings of our study and the previous reports, taken together, raise the necessity of more detailed studies to shed light on the mechanisms of ion absorption and transportation in the less-known primitive tetraploid wheat.

Salinity may exert both direct and indirect impacts on plants. One of the major indirect effects of salinity is the reduction in the photosynthetic capacity and, in turn, the imposition of indirect oxygen-induced cellular damages due to the increased ROS generation in the cells (Mittler 2002). Excessive amounts of highly reactive ROS can impose oxidative-induced damages to proteins, lipids, and nucleic acids (Halliwell and Gutteridge 1985). Hence, salt stress tolerance may depend at least in part on the capacity of the antioxidative defence system that is made up of the antioxidant compounds and several antioxidative enzymes. In plant cells, different antioxidative enzymes may take part in regulating the intracellular concentration of H_2O_2, though CAT, APX, POX, and glutathione reductase are considered the most prevalent ones. CAT is the principal scavenger of H_2O_2 in peroxisomes and eliminates H_2O_2 by its direct decomposition to water and molecular oxygen (Mittler 2002). Increase in CAT activity is thought to be an adaptive mechanism possibly helping to overcome the ROS-driven damage to the cells (Tunc-Ozdemir *et al.* 2009). In the present study, in contrast to HW, where CAT activity remained unchanged when treated by the S50, S100, and S150 (Table 4), the decrease in CAT activity of FTW excluded CAT as an effective scavenging mechanism to remove H_2O_2 in the latter genotype which could lead to cellular damage and, therefore, suppression of plant growth.

The role of APX and glutathione reductase in the H_2O_2 scavenging in the ascorbate-glutathione cycle of plant cells has been well established. The elevated glutathione reductase activity could increase the GSH/GSSG ratio, which is required for regeneration of ascorbate and activation of several CO_2-fixing enzymes in the chloroplasts (Mittler 2002), ensuring $NADP^+$ availability to accept electrons from the photosynthetic electron transport chain (Reddy *et al.* 2004). Moreover, ascorbate is the most common reducing substrate for H_2O_2 detoxification in plant cells and APX uses ascorbate to reduce the H_2O_2 to water (Noctor *et al.* 2002). In the present study, significant decrease in APX activities due to the salt stress seemed to be limited to FTW (Table 4).

HW indicated a consistently low APX activity under the concentrations of NaCl studied here. Similar to the APX activity, where HW and FTW behaved differently, the POX activity in HW and FTW appeared much different (Table 4). In fact, CAT, APX, and POX activities of FTW at the absence of salt were almost four to five times greater than that of HW. However, the substantial depression of the antioxidative activity in the salt-treated FTW plants, in contrary to minimal decreases in the salt-stressed HW, led to comparable activities of the latter enzymes in FTW and HW at the presence of S150. Many studies have confirmed the existence of differences in expression or activity of antioxidant enzymes; these differences have sometimes been associated with the more tolerant genotype and sometimes with the more sensitive genotype (Abogadallah 2010). Abogadallah (2010) and Munns and Tester (2008) reported that genetic differences in salinity tolerance are not necessarily due to differences in the ability to detoxify ROS. They suggested that differences in the antioxidant activity between genotypes may be due to genotypic differences in degrees of stomatal closure or in other responses that alter the rate of CO_2 fixation

Scavenging of ROS depends on both enzymatic and nonenzymatic components. Car are amongst the most important nonenzymatic antioxidants. Car, through receiving excess energy from the excited Chl, are able to quench singlet oxygen and decrease its formation (Abogadallah 2010). In most of the genotypes studied in this work, and particularly in the FTW genotype 'Yavaroos', the Car concentration significantly decreased with the increase in the NaCl concentration (Table 3), confirming the conclusion made by Abogadallah (2010) that contribution of Car to ROS scavenging is ambiguous. Car concentration of the stress-stricken tissue may decrease or increase depending on plant species (Sudhir and Murthy 2004). The salt-induced decrease of Car concentration in our study might be attributed either to a low synthesis rate or to enhanced degradation induced by ROS (Ashraf and Harris 2013). Decreases in Car concentration under salinity conditions have been reported in previous studies (Abogadallah 2010). The extent to which plants rely on each of the above defensive mechanisms including antioxidative enzyme activities may vary with rapidity, severity, and duration of the stress. As it has been hinted by Abogadallah (2010), the relative importance of each type of ROS scavenging activity (SOD, CAT, POX, *etc.*) differs between plants. Furthermore, efficient antioxidative activity does not necessarily mean the upregulation of the full set of antioxidant enzymes and *vice versa*. On the other hand, even though salt stress may lead to a rapid increase of ROS-scavenging enzyme activities, high salt concentrations may lead to a reduction or inhibition of the enzyme activities. We did not attempt to measure some of the foregone potent components of the defensive machinery in the tetraploid wheats, but the notable differences in the antioxidative enzyme activities between the salt-stressed plants of HW and FTW can be interpreted as evidence for differential stress-responding or adaptive behaviour of the two tetraploid wheat types.

The active accumulation of compatible solutes, such as amino acids, polyamines, and sugars, appears to be an effective mechanism of stress tolerance (Gilbert *et al.* 1998). Under stress conditions, plants set in motion the osmoregulatory functions and mechanisms by synthesizing compatible solutes including amino acid proline (Ashraf and Harris 2004). Proline is one of the most universal, polyfunctional, stress-protective substances, which accumulate in high concentrations when plants are exposed to salt stress (Fougere *et al.* 1991, Munns 2002). Proline accumulation in plant cells has been attributed to an increase in proline biosynthesis (Fougere *et al.* 1991), an increase in proteolysis or a decrease in protein synthesis (Ashraf and Harris 2004). Disagreements exist on the precise role played by compatible solutes (*e.g.,* proline) in plant's response to environmental stresses (Flowers *et al.* 2010). While it is often considered as a sink for excess nitrogen and taken as an osmolyte, proline can also confer enzyme protection and increase membrane stability under stressful conditions (Demiral and Türkan 2005). Proline has been also proposed as a signaling molecule initiating adaptation processes towards the stress and as an effective quencher of salt-induced ROS (Gill and Tuteja 2010). In our study, NaCl at 100 and 150 mM, and to a lesser extent at 50 mM concentration, led to increases in the leaf proline concentration in all genotypes (Table 3). HW did not appear significantly different from FTW, except at S100 (Table 4). Proline accumulation under saline conditions has been already reported in different cereal species including *T. durum* (Nabti *et al.* 2010), rice (*Oryza sativa* L.) (Demiral and Türkan 2005), and barley (*Hordeum vulgare* L.) (Widodo *et al.* 2009). The concentration of proline increased, more or less, in proportions to the Na^+ and Na^+/K^+ ratio and this proportionality appeared to be more notable for HW compared to FTW. Proline accumulation in the tetraploid wheat genotypes was well correlated to their Na^+ concentration and Na^+/K^+ ratio and production of this free amino acid in the HW was more notable at least when subjected to S100 (Table 4). It, therefore, seems that proline plays a protective role against moderate or severe salt stress in the hulled tetraploid wheat genotypes used in this experiment, confirming its previously proposed protective role (Fougere *et al.* 1991).

As far as osmotic effects of salt are concerned, inorganic ions, such as Na^+ and K^+, together with proline are known to be critical for osmotic adjustment in plant tissues. Therefore, a proportionally more notable tendency to maintain the osmoticum contents, *i.e.*, Na^+, K^+, and proline, in the salt-stricken HW, could be considered as evidence for the occurrence of some osmoregulatin in the latter primitive wheats compared to

FTW. This presumably greater capability of HW compared to FTW to cope with the osmotic effect of NaCl may have important implications in minimizing the deterioration of plant growth attributes subsequent to salt-induced osmotic stress. Considering the salt-induced 3.7-fold (HW) and 2.9-fold (FTW) enhancement of the proline concentration, we cannot exclude that this amino acid plays a ROS quenching role and helps relieve partially ionic and toxic effects of NaCl. Though, exact role of proline in response of HW and FTW to NaCl remains to be understood in future studies.

An inhibition of shoot and root growth is a common response to salinity and plant growth attributes are the most important agricultural criteria of salt stress tolerance (Munns 2002, Azizpour *et al.* 2010). As it has been emphasized by Munns (2002), salinity reduces the ability of plants for water uptake and also induces damage to the plant cells and tissues and these together, subsequently, lead to an array of physiological and metabolic changes and, therefore, reduction in a plant growth rate and dry mass. In addition to decrease in photosynthetic activity per unit of leaf area, excluding and/or compartmentalizing of salty ions within the cells brings about additional costs. Overall, SDM of HW tended to remain unchanged, in contrast to FTW, proving a less pronounced adverse effect of high salt concentrations (*i.e.,* 150 mM ≈ 15 dS m^{-1}) on the main growth attribute of HW (Table 4). It seems that the hulled tetraploid wheats studied here possess the capability to withstand, at least partially, the NaCl concentrations that are potentially detrimental to durum and even bread wheat. The observed reduction in SDM of FTW under saline condition might be the outcome of growth and physiological malfunctions, including the decrease in leaf expansion and leaf area, stomatal conductance, and photosynthetic rate (Munns and Tester 2008). Adverse effect of salinity on the plant biomass has been observed earlier across cereal crop species including *T. turgidum* (Munns and James 2003), *T. aestivum* (Ashraf and O'Leary 1996, Ashraf and McNeilly 1998), *O. sativa* (Chen *et al.* 2007), and maize (*Zea mays* L.) (Tuna *et al.* 2008). The insignificant effect of salt stress on RDM indicated that root growth was less affected by NaCl and that the root elongation rate recovered remarkably well after exposure to NaCl; these findings are in accordance with those reported by Munns and Tester (2008). In addition, roots have a remarkable ability to control Na^+ accumulation and also to regulate their turgor over a wide range of salinity levels than shoots. Thus, plant roots are expected to be more tolerant to salinity compared to the shoots (Munns *et al.* 2008).

Conclusion: Based on our findings, moderate concentrations of NaCl exhibited adverse effects on photosynthetic pigments, ionic, antioxidative and growth attributes of both types of tetraploid wheat. Since the hulled type of tetraploid wheat suffered less from the 150 mM NaCl compared to the free-threshing type of tetraploid wheat, it could be appreciated as a promising genetic resource for improving salt tolerance of *T. turgidum*. A tendency of the salt-stressed plants to accumulate proline, in contrary to the several-fold decrease or lack of increase in antioxidative response, might suggest a major role of this osmolyte at least in the hulled tetraploid wheats. Since a lesser reduction in SDM of hulled tetraploid wheats was not associated with their capacity to restrict Na^+ accumulation under saline conditions, such response can be viewed as an evidence for tissue tolerance in these less-utilized wheats. Further studies are needed to elucidate physiological basis of the difference, in response to salt, between *T. turgidum* and its hulled tetraploid relatives.

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