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REGULAR ARTICLE

Concurrence of ionic homeostasis alteration and dry mass sustainment in emmer wheats exposed to saline water: implications for tackling irrigation water salinity

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Abstract
Aims Over the course of plant breeding asset old resources such as emmer wheat have been neglected, therefore this study was carried out to evaluate usefulness of ancient emmer wheat genotypes in tackling agricultural soil and water salinity.
Methods Nine wheat genotypes consisting of four tetraploid (i.e. two standard durum and two emmer hulled wheats) and five hexaploid (i.e. three standard bread and two spelt-macha hulled genotypes) wheats were subjected to three irrigation water salinities (0, 60, and 120 mM NaCl).
Results Salinity imposed adverse effects on chlorophylls concentration, net CO_2 assimilation rate, stomatal conductance, sub-stomatal CO_2 concentration, shoot and root dry masses, and root volume of wheat groups examined. Salt-stricken plants of emmer and, to some extents, spelt-macha wheats displayed modest stability in chlorophylls and proline concentrations and shoot dry mass despite being out-performed in terms of net CO_2 assimilation rate and stomatal conductance by the durum and bread improved wheats. Na^+ concentrations and Na^+/K^+ of leaf sheath and blade were increased in all groups of wheat, but the magnitude of the increases in emmer and drum groups amounted to twice as much of those of the hexaploid wheats.
Conclusion Our novel finding was that the ionic imbalances and, contrariwise, dry mass stability and hence salt tolerance were evidently greater in the ancient emmer group of genotypes, compared to improved durum wheats.

Keywords Ancient cereal crops saline soil and water salt exclusion photosynthesis physiology

Abbreviations

Cars	Carotenoids
Chl	Chlorophyll
C_i	Sub-stomatal CO_2 concentration
FTW	Free threshing wheat
HW	Hulled wheat
F_0	Minimum Chl fluorescence
F_m	Maximum Chl fluorescence
F_v/Fm	Maximal quantum efficiency of photosystem II
g_s	Stomatal conductance to the CO_2
LPC	Leaf free proline concentration
LSD	Least significant difference
PCA	Principle component analysis
P_N	Net photosynthetic rate
RDM	Root dry mass
RWC	Relative water content
SDM	Shoot dry mass
V_{Root}	Root volume

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Introduction

Standard bread wheat (*Triticum aestivum* L.) is an allopolyploid species that has inherited its three genomes, i.e. A, B, and D from three different diploid species with Triticeae tribe (Shah et al. 1987). The A genome is known to have been derived from the einkorn diploid species of *Triticum monococcum*. The B genome has most probably been derived from a hybridization with wild diploid species of *Aegilops* or *T. speltoides*, giving shape to the wild emmer (*Triticum dicoccoides*) tetraploid wheat with AABB genome. The D genome is believed to have originated from a diploid species, namely *Triticum tauschii*. Inclusion of D genome has brought some crucially important traits to the standard hexaploid bread wheat. Larger leaves, better bread-making quality, and greater tolerance to salinity, compared to its wild and domesticated diploid and tetraploid relatives, are amongst the most distinguished traits (i.e. of the bread wheat) contributed by the D genome (Shah et al. 1987). All possible ploidy levels of *Triticum* species, including di-, tetra-, and hexaploids, could be found in the hulled type of wheats (Sheibanirad et al. 2014). The common names of the three main ancient hulled wheats are einkorn, emmer and spelt (and macha) for di-, tetra- and hexaploid levels, respectively. Ancient hulled wheats and landraces are thought to possess extensive variation in genes responsible for tolerance to biotic and some abiotic stresses (Moseman et al. 1985; Ruegger et al. 1990; Xie and Nevo 2008). The endemic landraces of hulled wheats of central Zagros in Iran are mainly tetraploid domesticated emmer (*Triticum turgidum spp. dicoccum*) containing AABB genome, as documented in a previous publication (Sheibanirad et al. 2014). Judging from the drastic declining trend in cultivation (i.e. at least in the Middle East and the Fertile Crescent) of this ancient tetraploid wheat type, it is imaginable that this species is prone to genetic erosion. An improved knowledge of the physiological traits of the endemic landraces of emmer wheat could be used as a potent tool for improving the stress tolerance of our present-day staple crops (e.g. bread and durum (*Triticum turgidum* L.) wheats), tackling the ever-increasing threat of salinity of soil and water resources and helping to solve, at least in part, the problem of genetic erosion and/or extinction of this somewhat neglected species.

Even though drought is the main environmental obstacle to agricultural productivity in arid and semi-arid regions, the already serious water salinity is predicted to become graver in the future as an outcome of decreased precipitation and increased evapo-transpiration due to climate change (Chamekh et al. 2015) and hence intensification of irrigation. Soil salinization is considered as a major man-made, though ecologically unsound, phenomenon around the world (Mcwilliam 1986). Saline soils are estimated to account for nearly 6, 20, and 50% of the world (Flowers 2004), cultivated land and irrigated land areas (Sudhir and Murthy 2004), respectively. Soil and irrigation water salinity brings serious harms to the plants of different species, unless the species possesses a certain level of tolerance against the salt-driven stresses. Salt-induced stress is rather a complex syndrome consisting of osmotic, ion toxicity, and nutrient deficiency stresses (Munns 2002; Tabatabaei and Ehsanzadeh 2016). Elevated concentrations of different inorganic ions and organic metabolites in the plant cells lead to a lowering of osmotic potential, necessitating more energy expenditures towards maintaining cell turgor and, hence, continuing water absorption from the soil at the expense of plant growth and SDM production. One of the most extensively studied responses of different plant species to the osmotic component of the salinity is the biosynthesis and accumulation of osmolytes, with amino acid proline being one of the most frequently detected molecules. Increased proline concentration is viewed as a counter-measure towards correcting plant water status, leaf relative water content (RWC), and water potential through triggering osmoregulation. Salt-induced accumulation of ions such as Na^+ leads to toxic and nutrition imbalance effects. Even though involvement of osmolytes such as proline in osmoregulating measures of salt-stressed plants has been confirmed, but playing such a role by these molecules in some species or genotypes could be by far smaller than the role played by inorganic ions, i.e. K^+ and Na^+. Controlling the soil salinization process and adopting new salt-tolerant crops and/or plant genetic resources are known as two critical approaches towards meeting the eminent need for the food resources of the growing global population (Läuchli and Luttge 2002).

Wheats of different levels of ploidy are thought to differ, among others, in terms of steadfastness against salinity. In fact, the *Kna1* gene which is known to be contributing to salt exclusion in wheat has been mapped to 4DL chromosome of bread wheat. This gene locus is therefore absent in the AB genome of tetraploid durum wheat and this wheat is more sensitive to salt, compared

to the hexaploid bread wheat (Shavrukov et al. 2009). Less-improved domesticated emmer relatives of durum wheat are thought to be equally tolerant to salinity but more tolerant to drought, compared to this wheat. Spelt and macha ancient hulled hexaploid wheats and Indian dwarf have ancestry relationships to the bread wheat, but information on their response to salinity is scanty. Indifference towards ancestors and relatives of durum and bread wheats has given us insufficient understanding of how and to what extent these genetic resources can lend us a hand to get along with the anticipated aggravation of salinity of our soil and water resources in the face of global climate change. This work aimed at examining and comparing physiological responses of some emmer, spelt, and macha ancient hulled wheats along with Indian dwarf, bread and durum improved wheat genotypes to irrigation water salinity.

Materials and methods

Plant material and growth conditions

Nine wheat genotypes including four tetraploid and five hexaploid genotypes were used to carry out this experiment. The experiment was carried out in a greenhouse at the Isfahan University of Technology (Latitude of 32° 38′ North, Longitude of 51° 39′ East, and an Altitude of 1656 m above sea level), Isfahan, Iran. The four tetraploid genotypes consisted of two emmer HW (*Triticum turgidum ssp. dicoccum*) genotypes and two durum FTW (*Triticum turgidum ssp. durum)* genotypes. The five hexaploid genotypes consisted of two ancient HW (a spelt (*Triticum aestivum ssp. spelta*) and a macha (*Triticum aestivum ssp. macha*) genotype), one Indian dwarf FTW (*Triticum aestivum ssp. sphaerococcum*), and two improved bread FTW (*Triticum aestivum*) genotypes. The two emmer wheat genotypes were Singerd and Joneghan. The two durum wheats were Yavaroos and TRI9652. The two ancient hulled hexaploid wheats were TRI3429 (spelt wheat) and TRI13595 (macha wheat) and the three bread wheat genotypes were Roushan, TRI19322, and the Indian dwarf TRI18664 (*T. sphaerococcum*). Further information on these genetic materials is given in Table 1. Seeds of these genotypes were surface sterilized with a 1% sodium hypochlorite, and planted in polyethylene-made containers (60 cm in height and 20.2 cm in diameter) containing 9000 ± 500 g of washed sand. These containers created 50 ± 3 cm tall soil columns. The bottom holes of the containers were insulated with gravels to avoid losses of the substrate. Rubber-made saucers were kept underneath the containers to prevent possible water loss due to drainage from the hole. The containers were watered with 700–1100 mL of either tap water or a Hoagland's solution (Hoagland and Arnon 1950) in each irrigation event over the course of experiment. Ten seeds were sown in each container and after establishment the seedlings were thinned to three. Seedlings were irrigated once with a half-strength Hoagland's nutrient solution from emergence to thinning. Thereafter, for preventing a nutrient deficiency in the washed sand medium, the plants were supplied with a full-strength Hoagland's solution following four events of tap water supplement. No salt treatment was carried out until the sown seeds were germinated and the plants had reached tillering, whereby saline solutions were applied into the containers in 30 mM increments in 2-days intervals until

Table 1 Genetic resources used in the experiment

Wheat type	Given name	Scientific name	Application		Origin
Hulled Tetraploid	Joneghan	*T. dicoccum*	Ancient emmer wheat	Emmer HW	Central Iran
Hulled Tetraploid	Singerd	*T. dicoccum*	Ancient emmer wheat	Emmer HW	Central Iran
Naked Tetraploid	TRI 9652	*T. turgidum*	Improved durum wheat	Durum FTW	IPK[a]
Naked Tetraploid	Yavaroos	*T. turgidum*	Improved durum wheat	Durum FTW	Iran
Hulled Hexaploid	TRI 3429	*T. spelta*	Ancient spelt wheat	Spelt HW	IPK
Hulled Hexaploid	TRI 13595	*T. macha*	Ancient macha wheat	Macha HW	IPK
Naked Hexaploid	TRI 18664	*T. sphaerococcum*	Indian dwarf wheat	Bread FTW	IPK
Naked Hexaploid	TRI 19322	*T. aestivum*	Improved bread wheat	Bread FTW	IPK
Naked Hexaploid	Roushan	*T. aestivum*	Improved bread wheat	Bread FTW	Iran

[a] IPK, Leibniz Institute of Plant Genetics and Crop Plant Research, Germany

the target salinity levels of either 60 or 120 mM were achieved. This cautious implementation of salt treatment was undertaken in order to avoid an osmotic chock. The control containers (i.e. containing non-stressed plants) were given either tap water or a non-saline Hoagland's solution, when necessary. Electrical conductivity and pH of the drained solution were measured and used for ascertaining sustained pH (6–6.5) and salinity levels for all experimental units, i.e. containers. Thus, leaching was carried out four times throughout the exertion of salinity treatment (i.e. tillering to dough stage). Plants were grown in outdoor condition from November 10 (seedling stage), 2016 to June 20, 2017 (physiological maturity stage). A total precipitation of 90 mm was received, absolute minimum and maximum temperatures were recorded to be − 9.8 and 39.8 °C, respectively, photoperiod varied from 10 to 16 h, and maximum photosynthetic photon flux density was recorded to be 1800 μmol m^{-2} s^{-1} over the course of experiment.

Measurement of chlorophyll and carotenoids concentrations

Chlorophyll (Chl) concentration was determined using fully expanded leaves. A fresh leaf sample of 200 mg was taken five weeks after implementing salt treatments, ground and extracted with 10 mL of 80% (*v*/v) acetone in the dark. The slurry was filtered, centrifuged (*5810R, Eppendorf Refrigerated Centrifuge*, Germany) at 5000×g for 10 min and absorbencies were determined at 645, 663 and 470 nm, for Chl *a*, Chl *b* and carotenoids (Cars) concentrations, respectively, using a spectrophotometer (*U-1800 UV/VIS, Hitachi*, Japan) and acetone (i.e. 80%) was used as blank. Concentrations of Chl *a*, Chl *b*, and Cars were determined and expressed as mg g^{-1} leaf fresh weight according to Lichtenthaler and Wellburn (1994).

Measurement of leaf gas exchange and chlorophyll fluorescence parameters

Net photosynthetic rate (P_N), stomatal conductance to the CO_2 (g_s), and sub-stomatal CO_2 concentration (C_i) were measured five weeks after application of NaCl on 3 youngest fully expanded flag leaves per container (i.e. experimental unit) with a calibrated portable gas exchange system (*LCi, ADC Bioscientific Ltd.,* UK) between 10:30 to 13:30. These measurements were carried out under photosynthetic photon flux density of 800–1200 μmol m^{-2} s^{-1}, ambient temperature of 26–31 °C, and ambient atmospheric CO_2 concentration of nearly 375 μmol mol^{-1}. A mean of the three measurements for each attribute was used for each container. Chlorophyll fluorescence parameters including maximum fluorescence (F_m) and minimum fluorescence (F_0) along with the maximum efficiency of photosystem II (F_v/Fm) were measured on three fully expanded young (dark-adapted for 20 min) attached flag leaves per container between 10:30 to 13:30 four weeks after NaCl imposition, using a portable chlorophyll fluorimeter (*OS-30p, Opti-Sciences, Inc.,* Hudson, NH, USA).

Measurement of proline concentration

Leaf free proline concentration (LPC) was determined according to Bates et al. (1973). 200 mg of fresh mature flag leaf sample was taken five weeks after implementing salt treatment, grinded in aqueous sulfosalicylic acid, the preparation of the mixture and the supernatant was undertaken as described in Tabatabaei and Ehsanzadeh (2016) and absorbance was measured at 520 nm by spectrophotometer. Toluene was used as blank. LPC was calculated using the standard curve prepared with known concentrations (e.g. 0 to 0.1 μg mL^{-1}) of this amino acid.

Measurement of root and shoot ion concentrations

The plant root and leaf sheath and blade samples of 500 mg were taken at harvest, the roots were washed with tap water, blotted-dry and ground, then leaf and root samples were ashed at 550 °C for 4 h, inorganic ions were extracted with 2 N hydrochloric acid (HCl), the Na^+, K^+ and Ca^{+2} concentrations were measured using a flame photometer (Corning Flame Photometer 410, Corning Medical and Scientific, Halstead Essex, UK) as described in Tabatabaei and Ehsanzadeh (2016).

Growth and rooting parameters

At physiological maturity (approximately 210 days after planting), roots and shoots were separated, washed, and blot dried. Root dry masses (RDM) were measured following rinsing off soil particles from the roots. Root volume (V_{Root}), RDM, and SDM of three plants per container were measured. Root volume was quantified by placing the roots into a measuring cylinder

containing a known volume of tap water. Dry masses were determined after the samples were oven-dried for 48 h at 70 °C.

Experimental design and statistical analysis

A factorial experiment comprised of NaCl at three concentrations and genotype at nine levels was conducted using a completely random design with three replications. Analysis of variance was carried out using Statistical Analysis Software version 8.2 (SAS Institute Inc., Cary, North Carolina, USA). Mean comparisons were conducted using Fisher's least significant difference (LSD) at $P \leq 0.050$. Principal component analysis (PCA) was carried out using STATGRAPHICS statistical software (16.2.04). This analysis was conducted to visualize the correlation patterns among performance and physiological traits of the examined wheat types in response to salt stress.

Results

Salinity significantly ($p \leq 0.01$) affected all examined traits (Table 2). Genotypes differed significantly ($p \leq 0.01$) in terms of all measured attributes, with the exception of F_v/Fm. The interaction effect of salinity × genotype was statistically significant ($p \leq 0.01$) for all of the traits with the exception of F_v/Fm. Because of the existence of the significant interactions, hereafter mean comparisons between the three levels of salt treatment, and the nine genotypes are not going to be explained. Instead, mean comparisons for interaction effect of salinity × genotype with emphasis on comparative responses of emmer HW, durum FTW, spelt-macha HW, and bread FTW groups to salinity are focused on, as the core purpose of this study was to shed light on the differential responses of wheats of different ploidy levels and ancient and improved wheats to salinity.

Gas exchange attributes, i.e. P_N, C_i, and g_s of all genotypes in emmer HW, durum FTW, spelt-macha HW, and bread FTW groups of wheat were decreased with increase in NaCl concentration of irrigation water (Table 3). The extent of the decreases was somewhat different among genotypes; emmer HW and spelt-macha FTW genotypes tended to be out-numbered (with the exception of C_i) by the remaining genotypes in durum FTW and bread FTW groups of wheat.

Photosynthetic pigments, i.e. Chl and Cars, responded to salinity in a genotype-specific manner. While concentrations of the pigments of the durum FTW group were increased at 60 mM NaCl, they remained unchanged at the 120 mM, compared to control plants (Table 3). In the meantime, none of these pigments in the Joneghan emmer HW was modified notably but they were decreased in Singerd emmer HW with increase in NaCl concentration. Furthermore, in contrary to decrease in concentration of the pigments in the bread FTW, concentration of these pigments were increased in the spelt HW but they were not modified notably in the macha HW, when exposed to NaCl.

Proline concentration of genotypes in durum and bread FTW groups increased with increase in NaCl, but that of the emmer and spelt-macha HW genotypes did not indicate such modifications (Table 3). While salt-induced modifications in LPC of emmer and spelt-macha HW genotypes were not found to be significant, increases in the durum and bread FTW genotypes were in the 200–300% range.

Root Na^+ concentration of all genotypes in different groups of wheat was increased with increase in NaCl, but increases in the genotypes of the two tetraploid groups (i.e. emmer and durum) were in the range of 22–60% and those of the genotypes in the two hexaploid groups (i.e. spelt-macha and bread) were in the 200–300% range. Leaf sheath and blade Na^+ concentrations of genotypes of all groups of wheat increased by several folds in the presence of 120 mM NaCl (Table 4). Nonetheless, leaf sheath and blade Na^+ concentrations of the genotypes in the two tetraploid groups (i.e. emmer HW and durum FTW) were more or less twice as much of those of the hexaploid groups (i.e. spelt-macha HW and bread FTW). Root and leaf sheath and blade K^+ concentrations of all genotypes in different groups of wheat were decreased and these decreases varied from 5% to 75% across the examined tissues and genotypes. While root K^+ concentration of the genotypes in the two tetraploid groups (emmer HW and durum FTW) were not notably different from those of the two hexaploid groups (spelt-macha HW and bread FTW), K^+ concentration of the leaf sheath and blade in the salt-stressed durum FTW and emmer HW groups amounted to only 40–67% of those of bread FTW and spelt-macha HW groups. Root Ca^{+2} concentration responded to NaCl in a wheat group-dependent manner. While root Ca^{+2} concentration of durum FTW, spelt-macha HW, and bread wheat FTW genotypes were mainly decreased, that of the emmer

Table 2 Analysis of variance (mean squares) for different traits of wheat genotypes evaluated at tree levels of irrigation water salinity (Salt) in 3 replications

	df	P_N	C_i	g_s	F_v/Fm	Chl		Cars	Proline	RDM	V_{Root}	SDM	Na^+_{Root}
Genotype	8	22.7**	5699**	0.0104**	0.00208**	0.436**		0.096**	0.316**	2.08**	54.4**	32.1**	0.0121**
Salt	2	25.4**	12877**	0.0317**	0.00005ns	0.472**		0.090**	0.792**	9.18**	200**	54.0**	0.0369**
Genotype×Salt	16	2.75**	425**	0.0011**	0.00019**	0.343**		0.088**	0.125**	0.54**	9.59**	2.70**	0.0030**
Error	60	0.568	111	0.00009	0.00003	0.0122		0.006	0.0165	0.033	1.21	0.35	0.0006
	df	Na^+_{Sheath}	Na^+_{Leaf}	K^+_{Root}	K^+_{Sheath}	K^+_{Leaf}	Ca^{+2}_{Root}	Ca^{+2}_{leaf}	Na^+/K^+_{Root}	Na^+/K^+_{Sheath}	Na^+/K^+_{Leaf}	Na^+/Ca^{+2}_{Root}	Na^+/Ca^{+2}_{Leaf}
Genotype	8	0.0889**	0.194**	0.010**	0.151**	0.076**	0.907**	0.0008**	0.133**	2.77**	7.76**	0.189**	78.0**
Salt	2	0.5108**	1.915**	0.017**	0.532**	0.572**	0.117**	0.0095**	0.442**	13.06**	59.2**	0.136**	2752**
Genotype×Salt	16	0.0328**	0.099**	0.004**	0.014**	0.033**	0.042**	0.0002**	0.195**	0.896**	3.99**	0.043**	66.5**
Error	60	0.0010	0.0023	0.0004	0.0032	0.0035	0.0099	0.00006	0.0199	0.0722	0.121	0.0105	8.89

df, degrees of freedom; P_N, Net photosynthetic rate; C_i, substomatal CO_2 concentration; g_s, stomatal conductance to the CO_2; F_v/Fm, maximum quantum efficiency of the photosynthetic photosystemII; Chl_{total}, total chlorophyll concentration; *Cars*, carotenoids concentration; *RDM*, root dry mass; SDM: shoot dry mass; *V*, volume; ns: non-significant

* $p \leq 0.05$; ** $p \leq 0.001$

Table 3 Mean comparisons of Wheat genotype × Salt interaction for different photosynthetic and physiological traits of nine wheat genotypes evaluated at three levels of irrigation water salinity (Salt)

Traits	P_N			C_i			g_s			F_v/Fm		
Treatments	[μmol CO_2 m^{-2} s^{-1}]			[μmol CO_2 mol^{-1}]			[mol CO_2 m^{-2} s^{-1}]					
Salt(mM)	0	60	120	0	60	120	0	60	120	0	60	120
Wheat Genotype												
Joneghan	10.6^{ghi}	10.6^{ghi}	8.7^{klm}	168^{ab}	142^{e}	90^{ij}	0.157^{d}	0.127^{fg}	0.077^{lmn}	0.761^{mno}	0.775^{g-k}	0.766^{klm}
Singerd	10.7^{ghi}	10.8^{gh}	8.6^{lm}	113^{f}	94^{g-j}	93^{hij}	0.110^{hi}	0.099^{ijk}	0.073^{mn}	0.780^{f-i}	0.778^{f-j}	0.781^{e-h}
TRI 9652	10.2^{hij}	10.2^{hij}	9.2^{jkl}	143^{e}	119^{f}	85^{jk}	0.120^{gh}	0.100^{ij}	0.067^{n}	0.758^{no}	0.770^{jkl}	0.765^{lmn}
Yavaroos	15.0^{a}	13.7^{bc}	10.9^{gh}	119^{f}	90^{ij}	81^{jk}	0.173^{c}	0.123^{gh}	0.090^{jkl}	0.785^{ef}	0.783^{efg}	0.771^{i-l}
TRI 3429	9.9^{h-k}	9.5^{il}	7.6^{m}	181^{a}	161^{bcd}	144^{de}	0.150^{de}	0.123^{gh}	0.083^{klm}	0.803^{b}	0.801^{bc}	0.789^{de}
TRI 13595	11.8^{efg}	12.2^{def}	11.8^{efg}	117^{f}	109^{fgh}	69^{k}	0.127^{fg}	0.127^{fg}	0.097^{ijk}	0.765^{lmn}	0.780^{mno}	0.755^{o}
TRI 18664	14.1^{a}	12.5^{cde}	10.7^{ghi}	148^{cde}	110^{fgh}	105^{f-i}	0.207^{b}	0.127^{fg}	0.103^{ij}	0.783^{ef}	0.766^{lmn}	0.772^{i-l}
TRI 19322	11.0^{fpg}	10.4^{hij}	12.2^{def}	164^{abc}	121^{f}	111^{fg}	0.163^{cd}	0.120^{gh}	0.130^{fg}	0.773^{h-l}	0.794^{cd}	0.798^{bc}
Roushan	15.1^{a}	13.4^{bcd}	11.7^{efg}	167^{ab}	171^{ab}	148^{cde}	0.270^{a}	0.213^{b}	0.140^{ef}	0.812^{a}	0.798^{bc}	0.805^{ab}
LSD		1.23			17.3			0.015			0.009	
Traits	Chl			Cars			Proline			SDM		
Treatments	[mg g^{-1}]			[mg g^{-1}]			[μmol g^{-1}]			[g]		
Salt(mM)	0	60	120	0	60	120	0	60	120	0	60	120
Wheat Genotype												
Joneghan	2.01^{def}	2.04^{def}	1.92^{fg}	0.371^{d-g}	0.407^{cde}	0.310^{e-j}	0.215^{i-m}	0.383^{e-j}	0.392^{e-i}	8.65^{bcd}	8.96^{bc}	7.43^{fg}
Singerd	1.90^{fg}	1.55^{ijk}	1.52^{i-l}	0.326^{e-i}	0.155^{k-o}	0.158^{k-o}	0.235^{i-m}	0.197^{klm}	0.322^{g-m}	7.59^{efg}	8.92^{bc}	8.08^{e-f}
TRI 9652	1.41^{klm}	1.69^{hi}	1.61^{ij}	0.132^{l-o}	0.206^{i-n}	0.190^{j-o}	0.350^{f-l}	0.543^{cde}	1.147^{a}	8.45^{b-e}	9.31^{b}	4.60^{j}
Yavaroos	1.80^{gh}	2.32^{a}	1.34^{lmn}	0.275^{f-k}	0.620^{a}	0.102^{mno}	0.223^{i-m}	0.685^{c}	0.943^{b}	4.41^{j}	4.33^{j}	1.67^{l}
TRI 3429	1.50^{jkl}	1.68^{hi}	2.33^{a}	0.155^{k-o}	0.227^{h-l}	0.629^{a}	0.220^{i-m}	0.468^{d-g}	0.279^{h-m}	8.65^{bcd}	10.58^{a}	5.08^{hij}
TRI 13595	2.07^{c-f}	2.23^{abc}	2.07^{c-f}	0.401^{c-f}	0.565^{ab}	0.418^{cde}	0.206^{j-m}	0.171^{lm}	0.256^{h-m}	7.42^{fg}	7.72^{d-g}	4.54^{j}
TRI 18664	2.11^{b-e}	1.24^{mno}	1.20^{no}	0.475^{bcd}	0.089^{no}	0.065^{o}	0.176^{lm}	0.425^{e-h}	0.505^{c-f}	5.71^{hi}	4.98^{ij}	2.84^{k}
TRI 19322	2.25^{ab}	2.18^{a-d}	1.80^{gh}	0.570^{ab}	0.506^{abc}	0.254^{g-l}	0.307^{g-m}	0.369^{e-k}	0.230^{i-m}	7.00^{g}	6.03^{h}	5.14^{h-j}
Roushan	1.98^{ef}	1.96^{efg}	1.11^{o}	0.353^{d-h}	0.340^{e-h}	0.062^{o}	0.164^{m}	0.653^{c}	0.640^{cd}	5.20^{hij}	4.34^{j}	2.72^{k}
LSD		0.181			0.129			0.210			0.970	

Within each trait, means followed with the same letters are those with differences less than LSD, i.e. do not have statistically significant differences at 5% level of probability

P_N, Net photosynthetic rate; C_i: substomatal CO_2 concentration; g_s, stomatal conductance to the CO_2; F_v/Fm, maximum quantum efficiency of the photosynthetic photosystem II; Chl_{total}, total chlorophyll concentration; *Cars*, carotenoids concentration; *SDM*, shoot dry mass; *LSD*, least significant difference

HW group was not altered significantly with increase in NaCl concentration. In fact, salt-treated plants of emmer HW group genotypes out-numbered the genotypes in remaining groups in terms of root Ca^{+2} concentration. Leaf Ca^{+2} concentration of genotypes in all examined wheat groups were decreased more or less in a same way and extent, when grown in the presence of NaCl, but salt-stricken emmer HW and durum FTW tended to contain greater amounts of Ca^{+2}, compared to spelt-macha HW and bread FTW. Leaf sheath and blade Na^+/K^+ of all genotypes in different groups of wheats were increased by several-fold, when grown in the presence of 120 mM NaCl. Meanwhile, leaf sheath and blade Na^+/K^+ in the salt-stricken plants of the two tetraploid wheat groups (emmer HW and durum FTW) were 2 to 3-fold greater than those of the spelt-macha HW and bread FTW groups (Table 5). While root Na^+/K^+ of emmer HW, durum FTW, and bread FTW genotypes remained more or less unchanged, that of the spelt-macha HW genotypes was increased with increase in NaCl concentration. Root Na^+/Ca^{+2} of bread wheat genotypes was increased with increase in NaCl concentration, but emmer HW, durum FTW, and spelt-macha HW genotypes did not indicate such increases. Leaf

Table 4 Mean comparisons of Wheat genotype × Salt interaction for different root and ionic traits evaluated at tree levels of irrigation water salinity (Salt)

Traits	V_{Root}			RDM			Na^+_{Root}			Na^+_{Sheath}			Na^+_{Leaf}		
Treatments	[cm^3]			[g]			[mmol g^{-1}]			[mmol g^{-1}]			[mmol g^{-1}]		
Salt(mM)	0	60	120	0	60	120	0	60	120	0	60	120	0	60	120
Genotype															
Joneghan	11.6^{def}	10.0^{fgh}	7.9^{ijk}	3.15^{b}	1.93^{fg}	1.45^{h-k}	0.073^{ghi}	0.122^{def}	0.129^{de}	0.093^{lmn}	0.383^{d}	0.581^{b}	0.073^{l-o}	0.510^{e}	1.201^{a}
Singerd	9.0^{hij}	13.4^{bc}	7.9^{ijk}	1.69^{gh}	2.63^{cd}	1.34^{jk}	0.087^{f-i}	0.093^{e-i}	0.108^{d-g}	0.114^{kl}	0.338^{de}	0.497^{c}	0.073^{l-o}	0.420^{fg}	0.609^{d}
TRI 9652	13.1^{bcd}	12.3^{cde}	7.1^{kl}	2.74^{c}	2.36^{de}	1.49^{h-k}	0.067^{hi}	0.074^{ghi}	0.086^{f-i}	0.061^{mno}	0.170^{ij}	0.670^{a}	0.055^{mno}	0.223^{ij}	1.025^{b}
Yavaroos	6.7^{klm}	5.1^{mno}	2.8^{op}	1.30^{jk}	1.50^{h-k}	0.70^{l}	0.073^{ghi}	0.145^{cd}	0.107^{d-g}	0.093^{lmn}	0.153^{ijk}	0.254^{fg}	0.145^{kl}	0.294^{hi}	0.485^{ef}
TRI 3429	14.2^{b}	13.1^{bcd}	5.0^{mno}	3.49^{a}	2.16^{ef}	0.90^{l}	0.055^{i}	0.122^{def}	0.122^{def}	0.079^{lo}	0.129^{jkl}	0.233^{gh}	0.049^{mno}	0.253^{hi}	0.484^{ef}
TRI 13595	10.8^{e-h}	11.2^{efg}	5.8^{lmn}	1.59^{hij}	1.39^{ijk}	0.69^{l}	0.055^{i}	0.188^{b}	0.145^{cd}	0.049^{no}	0.107^{klm}	0.178^{ij}	0.038^{mno}	0.153^{jk}	0.283^{hi}
TRI 18664	7.4^{jkl}	4.3^{nop}	3.6^{op}	1.30^{jk}	0.85^{l}	0.66^{l}	0.055^{i}	0.061^{i}	0.100^{e-h}	0.038^{o}	0.196^{hi}	0.305^{ef}	0.027^{o}	0.383^{g}	0.718^{c}
TRI 19322	11.0^{efg}	9.6^{ghi}	6.2^{klm}	2.22^{ef}	1.65^{ghi}	0.81^{l}	0.093^{e-i}	0.170^{bc}	0.179^{bc}	0.049^{no}	0.100^{lmn}	0.130^{jkl}	0.107^{k-n}	0.114^{klm}	0.253^{hi}
Roushan	16.7^{a}	11.1^{efg}	7.6^{jkl}	2.11^{ef}	1.67^{ghi}	1.25^{jk}	0.086^{f-i}	0.196^{b}	0.284^{a}	0.038^{o}	0.114^{kl}	0.205^{ghi}	0.032^{no}	0.170^{jk}	0.305^{h}
LSD		1.80			0.298			0.0385			0.0527			0.0778	
Traits	K^+_{Root}			K^+_{Sheath}			K^+_{Leaf}			Ca^{+2}_{Root}			Ca^{+2}_{Leaf}		
Treatments	[mmol g^{-1}]			[mmol g^{-1}]			[mmol g^{-1}]			[mmol g^{-1}]			[mmol g^{-1}]		
Salt(mM)	0	60	120	0	60	120	0	60	120	0	60	120	0	60	120
Genotype															
Joneghan	0.072^{e-i}	0.115^{cd}	0.121^{c}	0.506^{cd}	0.212^{hij}	0.220^{hi}	0.539^{cde}	0.212^{klm}	0.234^{jkl}	1.365^{a}	1.358^{a}	1.456^{a}	0.090^{a}	0.035^{fgh}	0.025^{h-l}
Singerd	0.084^{d-h}	0.090^{c-g}	0.103^{cde}	0.497^{cd}	0.205^{hij}	0.183^{ij}	0.515^{def}	0.212^{klm}	0.134^{mn}	0.905^{cd}	0.756^{d-g}	0.742^{e-h}	0.060^{c}	0.032^{f-j}	0.035^{fgh}
TRI 9652	0.067^{f-i}	0.073^{e-i}	0.084^{d-i}	0.556^{bc}	0.291^{fgh}	0.258^{ghi}	0.645^{b}	0.253^{jkl}	0.161^{lmn}	1.120^{b}	0.788^{def}	0.878^{de}	0.073^{b}	0.040^{def}	0.032^{f-j}
Yavaroos	0.072^{e-i}	0.115^{cd}	0.090^{c-g}	0.389^{e}	0.175^{ij}	0.122^{j}	0.445^{efj}	0.275^{ijk}	0.115^{n}	0.423^{k-n}	0.482^{j-n}	0.527^{i-m}	0.048^{cde}	0.033^{f-i}	0.027^{g-l}
TRI 3429	0.096^{c-f}	0.074^{e-i}	0.061^{ghi}	0.789^{a}	0.525^{bc}	0.425^{de}	0.622^{bc}	0.369^{ghi}	0.242^{jkl}	1.047^{bc}	0.603^{g-j}	0.780^{def}	0.037^{e-h}	0.020^{jkl}	0.017^{l}
TRI 13595	0.056^{hi}	0.168^{b}	0.096^{c-f}	0.601^{b}	0.379^{ef}	0.360^{ef}	0.776^{a}	0.407^{gh}	0.370^{ghi}	0.580^{h-k}	0.558^{i-l}	0.433^{k-n}	0.050^{cd}	0.028^{f-l}	0.022^{i-l}
TRI 18664	0.056^{hi}	0.050^{i}	0.096^{c-f}	0.334^{efg}	0.243^{ghi}	0.258^{ghi}	0.325^{hij}	0.316^{hij}	0.283^{ijk}	0.395^{mn}	0.602^{g-j}	0.447^{j-n}	0.082^{ab}	0.040^{def}	0.025^{h-l}
TRI 19322	0.078^{e-i}	0.161^{b}	0.169^{b}	0.823^{a}	0.568^{bc}	0.380^{ef}	0.537^{cde}	0.526^{cde}	0.325^{hij}	1.042^{bc}	0.743^{d-g}	0.680^{f-i}	0.038^{d-g}	0.020^{jkl}	0.018^{kl}
Roushan	0.084^{d-i}	0.176^{b}	0.258^{a}	0.387^{e}	0.370^{ef}	0.291^{fgh}	0.465^{efg}	0.600^{bcd}	0.425^{fg}	0.402^{lmn}	0.345^{n}	0.325^{n}	0.060^{c}	0.030^{f-k}	0.022^{i-l}
LSD		0.0338			0.0923			0.0963			0.163			0.0131	

Within each trait, means followed with the same letters are those with differences less than LSD, i.e. do not have statistically significant differences at 5% level of probability

V, volume; *RDM*, root dry mass; *LSD*, least significant difference

Na^+/Ca^{+2} in genotypes of all groups of wheat was increased drastically and there was a tendency in salt-stressed plants of emmer HW and durum FTW to out-number some of the spelt-macha HW and bread FTW genotypes in this regard.

Root dry mass and V_{Root} of all genotypes in different groups of wheat were decreased, when exposed to 120 mM NaCl (Table 4). Nonetheless, emmer HW group of wheats out-numbered durum FTW, bread FTW, and spelt-macha HW groups in terms of RDM and V_{Root}, when grown under saline condition, as the extent of salt-induced decreases were smaller in these genotypes. Shoot dry mass of emmer HW genotypes remained unchanged but those of the durum FTW, spelt-macha HW, and bread FTW genotypes were decreased substantially when treated with 120 mM NaCl; salt-exposed emmer HW plants out-numbered the durum FTW, spelt-macha HW, and bread FTW in this attribute.

According to the PCA and bi-plot analyses, the first two principal components (i.e. PC1 and PC2) accounted for 36.8% and 19.6% of the total variance, respectively, together explaining 56.3% of the total variance (Fig. 1). The PC1 was positively correlated with P_N, C_i, g_s, F_v/Fm, Chl, Cars, SDM, RDM, V_{Root}, and K^+ concentration of leaf but it was negatively correlated with LPC and Na^+ concentrations and Na^+/K^+ of all plant organs; hence, this component could be named photosynthesis-dry mass component. The PC2 was positively correlated with SDM, RDM, V_{Root}, Na^+ leaf, K^+ leaf, and Na^+/K^+ leaf but it indicated negative correlations with the remaining of the examined attributes; therefore, this component was distinguished as dry mass-ionic component.

Discussions

Even though SDM, RDM (hence, total plant dry mass), and V_{Root} tended to decrease in response to salt across all wheat groups, but the magnitude of the depressions in these attributes varied with wheat group and were in the descending order of durum FTW > bread FTW = spelt-macha HW > emmer HW (Tables 3 and 4). In fact, the present group of emmer HW out-numbered the remaining wheat groups in terms of RDM, V_{Root}, SDM, and total plant dry mass (data not shown) at least when grown under saline condition. Presented data are in line to the findings of Chamekh et al. (2015), as they found that landraces out-number the improved genotypes in terms of plant height and above-ground dry mass. Our findings agree to those of Badridze et al. (2009), where they found that Georgian endemic hexaploid *T. macha* and tetraploid *T. timopheevii* and *T. dicoccum* genotypes out-performed other tetraploid and hexaploid genotypes in terms of withstanding salinity at germination stage.

A suppressed plant performance (i.e. exemplified in notable decreases of RDM, V_{Root}, and SDM of the salt-stricken wheat plants of the present study) may arise from osmotic, toxic, and/or ion imbalance consequences of the salt. It appears from the data presented herein that NaCl induced these three components of salt damage in different degrees in the examined groups of wheat. Osmotic effects of salt occur, often, in association with modifications in plant organic and inorganic solutes concentrations. Even though proline accumulation has been attributed to an array of stresses and observed in all types of plants, but it must be understood that this osmolyte is not alone in tackling a stress. It is a metabolically expensive molecule that its accumulation is correlated to plant nitrogen status (Carillo 2018), i.e. its synthesis and accumulation is enhanced with increase in available nitrogen. Furthermore, other osmolytes such as γ-aminobutyric acid (GABA) and glycine betaine (Carillo 2018) and Na^+, sucrose, and asparagine (Annunziata et al. 2017) have been found to outnumber proline in salt-stressed durum wheat under certain environmental circumstances. Moreover, reliance of salt-stricken plants on compatible solutes (e.g. proline) and potentially toxic ions (e.g. Na^+) for osmoregulation may differ within a species and with the duration of salt exposure. For example, in contrary to *Hordeum vulgare*, the wild barley species *H. maritimum* relies on a delayed Na^+ accumulation at the early weeks of exposure to NaCl but invests in proline accumulation when faced with a prolonged salinity (Ferchichi et al. 2018). From the observation of merest modifications in LPC (Table 3) of the present emmer HW group (and in a lesser extent spelt-macha HW genotypes), and available literature on lack of association, i.e. in durum and emmer wheats, between drought tolerance and proline accumulation (Chandrasekar et al. 2000), we propose that these ancient wheats do not rely much on proline for tackling osmotic effects of saline irrigation water. Instead, substantially greater increases in leaf Na^+ concentration of salt-stressed tetraploid wheats, in general, and the emmer HW group, in particular (Table 4), lead us to propose that these less-known group of wheats may have benefited from inorganic ions as an inexpensive

means (Annunziata et al. 2017) to accomplish osmoregulation when grown in the presence of 120 mM NaCl. This minimal accumulation of proline is related, at least in part, to the accumulation of Na^+ in the presence of salt. In fact, Na^+ accumulation in the salt-exposed plants is not always detrimental (Bendaly et al. 2016), and findings of some studies such as those of Annunziata et al. (2017), Hariadi et al. (2011) and Shalata and Tal (1998) indicate that accumulation of inorganic ions, including K^+, Na^+ and Cl^- may play a greater role in osmotic adjustment, compared to the proline. Albeit, potent positive function of Na^+ to osmotic adjustment in salt-affected plant's areal tissue is fulfilled only if Na^+ is sequestered in the vacuoles to avoid alteration in cytoplasmic metabolic processes.

Further to the osmotic consequences of salt stress discussed above, increased concentration of Na^+ and Cl^- brings about nutrient imbalance and ion toxicity damages (Munns and Tester 2008: Cuin et al. 2009). Due to the considerable level of similarity in physicochemical structures of Na^+ and K^+, the former cation is potent to compete with K^+ in entering symplastic components, leading to a K^+ deficiency in cells of salt-stricken plants (Maathuis and Amtmann 1999). K^+ deficiency is harmful to some key cell functions. For instance, a high concentration of K^+ in the stroma of chloroplast is necessary to ensure a satisfactory operating of photosynthetic apparatus. Moreover, according to the findings of Li et al. (2010), substitution of cell membrane-bound Ca^{2+} by salinity-derived Na^+ could negatively affect the membrane permeability. From the notable decreases in K^+ and Ca^{+2} concentrations of different plant organs of the examined groups of wheat (Table 4), it is reasonable to surmise that the salt-induced increase in Na^+ concentration has led to interference in the absorption of K^+ and Ca^{+2} and the degree of this interference seems to be different among the studied groups of wheats. In fact, a tendency of some of the emmer and durum tetraploid wheat gentoypes of the present study to accumulate Na^+ in expense of Ca^{+2} and K^+ is in line with the finding of Cuin et al. (2009), where they concluded that durum wheat genotypes outnumber bread wheats in terms of intrinsic K^+ and Na^+ concentrations. An interesting finding of the present study was that the decreases in leaf Ca^{+2} concentration of salt-stricken tetraploid wheats, in general, and emmer HW wheats, in particular, tended to be smaller than that of the hexaploid groups of wheat (Table 4). Ion toxicity damages are negatively correlated to salt exclusion capability of the salt-exposed plants. Salt exclusion may be accomplished either at root, aerial organs, or cell compartment levels. A large proportion of the Na^+ is potentially removed by the root epidermis, outer cortical cell, pericycle, and xylem parenchyma from the transpiration stream of plant (Lauchli et al. 2008). A second potent line of exclusion of Na^+ and preventing it from reaching the leaves is the retrieval of Na^+ that enters the xylem in some parts of the roots. As it has been proposed by Matsushita and Matoh (1991) in their work on salt-tolerant reed, downward Na^+ transport from shoot base to root cannot be ruled out at least in the hexaploid wheat groups examined here. Evidence in support of the proposal of accomplishing salt exclusion at the root level (i.e., either through preventing Na^+ from entering xylem or retrieving Na^+ from xylem to the root) of the examined wheats of present study comes from the nearly 2 to 3-fold increased Na^+ accumulations in the roots of hexaploid wheats (that harbour the D genome) grown in the presence of 120 mM NaCl (Table 4).

It is believed that contribution of the D genome to salt tolerance in hexaploid wheats is accomplished mainly through a cation selectivity capability, i.e. discriminating against Na^+ and in favour of K^+ (Shah et al. 1987). Unlike hexaploid wheats, in general, and bread FTW, in particular, and judging from the ratio of Na^+/K^+, neither ancient emmer HW nor improved durum FTW groups appeared to benefit from a mechanism to establish a small Na^+/K^+ ratio (Table 5). Since tetraploid wheats do not possess the D genome to contain the *Kna1* gene controlling the Na^+/K^+ ratio, the increased Na^+/K^+ in the two groups of tetraploid wheat was not far from anticipation. In fact, involvement of *Nax1* and *Nax2* loci in salt tolerance of hexaploid wheat is thought to have been met by the function of genes of HKT family that prevent transport of Na^+ (and stimulate transport of K^+) from leaf sheath to leaf blade. Indirect evidence in favour of functioning of such gene family in the two hexaploid wheat groups of the present study is obtained from maintaining a greater K^+ concentration and hence a smaller Na^+/K^+ in the leaf blades under saline condition, compared to the two tetraploid wheat groups (Table 5).

Our examinations indicated differential modifications in Chl and Cars concentrations of the stressed plants (Table 3) which, albeit, decreasing ones did not come as a surprise, as 70–80% decreases in Chl concentration in salt-stressed plants have been reported (Bendaly et al. 2016). Substantial decreases in Chl and particularly Cars concentrations of the bread FTW group were contrasted by smaller decreases in the emmer HW group and significant increases in the

Table 5 Mean comparisons of Wheat genotype × Salt interaction for ionic ratios evaluated at tree levels of irrigation water salinity (Salt)

Traits	Na^+/K^+_{Root}			Na^+/K^+_{Sheath}			Na^+/K^+_{Leaf}			Na^+/Ca^{+2}_{Root}			Na^+/Ca^{+2}_{Leaf}		
Treatments															
Salt(mM)	0	60	120	0	60	120	0	60	120	0	60	120	0	60	120
Wheat Genotype															
Joneghan	1.01^{de}	1.06^{cde}	1.07^{cde}	0.186^{ghi}	1.830^{bc}	2.679^{a}	0.141^{ij}	2.519^{d}	5.158^{b}	0.098^{g-j}	0.123^{f-j}	0.148^{f-j}	1.23^{jkl}	13.78^{d-g}	17.52^{bcd}
Singerd	1.03^{de}	1.03^{de}	1.05^{cde}	0.242^{ghi}	1.679^{c}	2.769^{a}	0.142^{ij}	2.015^{d}	4.657^{bc}	0.062^{ij}	0.149^{f-j}	0.165^{e-g}	2.02^{jkl}	7.14^{hi}	19.78^{b}
TRI 9652	1.00^{de}	1.01^{de}	1.03^{de}	0.111^{hi}	0.605^{efg}	2.766^{a}	0.085^{j}	0.943^{ef}	6.389^{a}	0.060^{j}	0.093^{hij}	0.100^{g-j}	0.75^{kl}	5.40^{h-k}	33.59^{a}
Yavaroos	1.01^{de}	1.27^{c}	1.21^{cde}	0.241^{ghi}	0.886^{de}	2.225^{b}	0.327^{g-j}	1.076^{e}	4.229^{c}	0.378^{c}	0.318^{cde}	0.184^{e-j}	3.15^{i-l}	9.12^{gh}	19.57^{bc}
TRI 3429	0.58^{f}	1.64^{b}	2.00^{a}	0.102^{i}	0.247^{ghi}	0.549^{e-h}	0.080^{j}	0.683^{e-i}	1.998^{d}	0.053^{j}	0.207^{d-j}	0.156^{e-j}	1.35^{jkl}	12.63^{efg}	29.26^{a}
TRI 13595	0.99^{e}	1.12^{cde}	1.51^{b}	0.084^{i}	0.286^{f-i}	0.494^{e-i}	0.049^{j}	0.387^{f-j}	0.783^{efg}	0.096^{hij}	0.353^{d-j}	0.283^{e-j}	0.84^{jkl}	5.38^{h-k}	13.16^{d-g}
TRI 18664	0.99^{e}	1.21^{cde}	1.04^{cde}	0.118^{hi}	0.832^{de}	1.186^{d}	0.085^{j}	1.236^{e}	2.531^{d}	0.141^{f-j}	0.092^{hij}	0.230^{c-h}	0.34^{l}	9.78^{fgh}	30.04^{a}
TRI 19322	1.23^{cd}	1.06^{cde}	1.07^{cde}	0.060^{i}	0.180^{ghi}	0.355^{f-i}	0.209^{hij}	0.221^{g-j}	0.782^{efg}	0.077^{hij}	0.240^{c-h}	0.265^{c-g}	2.95^{i-l}	5.38^{h-k}	14.25^{def}
Roushan	1.03^{de}	1.13^{cde}	1.10^{cde}	0.098^{i}	0.311^{f-i}	0.717^{ef}	0.071^{j}	0.282^{g-j}	0.722^{e-h}	0.228^{c-i}	0.567^{b}	0.878^{a}	0.55^{kl}	5.65^{hij}	14.78^{cde}
LSD		0.231			0.440			0.570			0.168			4.88	

Within each trait, means followed with the same letters are those with differences less than LSD, i.e. do not have statistically significant differences at 5% level of probability

LSD, least significant difference

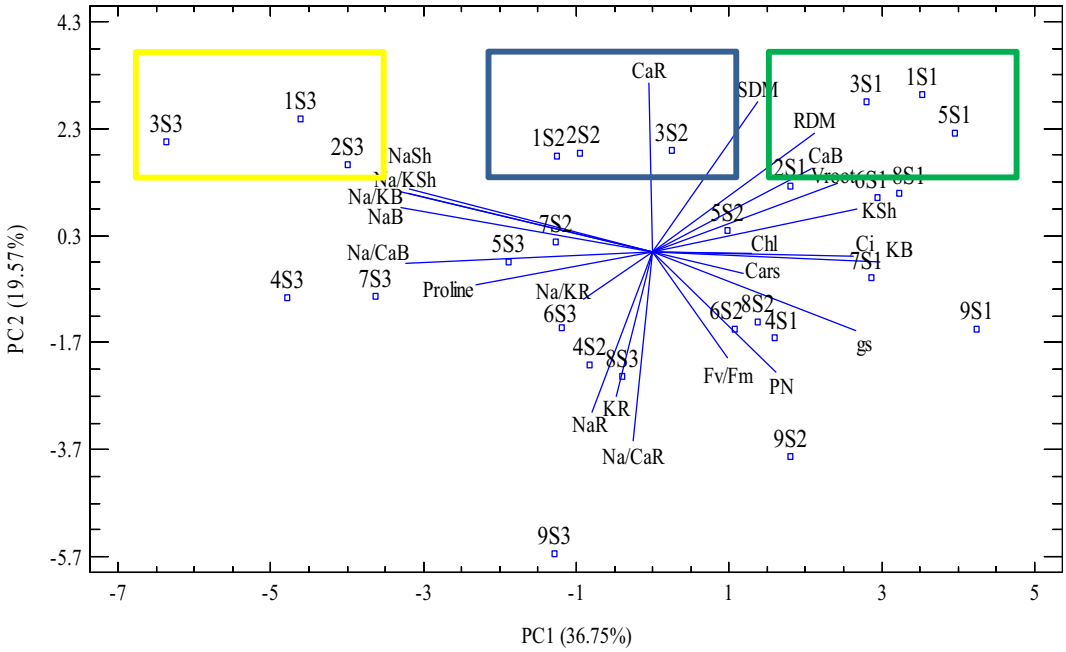

Fig. 1 Principle component analysis of physiological and performance attributes of emmer, durum, spelt, macha, and bread wheat genotypes in response to saline irrigation water. Numbers 1 to 9 stand for Singerd, Joneghan (emmer wheats), TRI9652, Yavaroos (durum wheats), TRI3429 (spelt wheat), TRI13595 (macha wheat), TRI18664 (Indian dwarf bread wheat), TRI19322, and Roushan (bread wheats), respectively. S1, S2, and S3 are indicative of control (no salinity), 60, and 120 mM NaCl, respectively. Vroot, R, Sh, and B indicate root volume, root, leaf sheath and blade, respectively. PN, gs, Ci, Chl, Car, and Fv/Fm indicate net photosynthetic rate, stomatal conductance to the CO_2, substomatal CO_2 concentration, chlorophyll concentration, carotenoids concentration, and maximum efficiency of photosystem II, respectively

concentration of these pigments in the spelt HW plants. A tendency in the durum FTW, emmer HW, and spelt-macha HW groups to maintain (and in some cases increase) the Cars and Chl concentrations in the presence of 60 and more importantly 120 mM NaCl (Table 3) seems to be, at least in part, in association to the declination of the ancient emmer HW and spelt HW to rely on proline synthesis and accumulation in the presence of salt. Both Chl and proline biosynthetic pathways are dependent on glutamine as a precursor. Hence, reliance of the stressed plants on the amino acid proline (as it has occurred in durum and bread wheat FTW groups in the present study) may potentially divert the photoassimilates towards this protective osmolyte in expense of photosynthetic pigments. The increased Chl concentration of the stressed spelt HW plants is, at least in part, related to a stress-induced increase in chloroplast concentration per mesophyll cell (Bazrafshan and Ehsanzadeh 2014). It is thought that stress-induced reactive oxygen species elicit carotenognesis via affecting redox state of electron carriers (e.g. plastoquinone) in the chloroplastic electron transport chain (Solovchenko and Neverov 2017). Therefore, a tendency of salt-exposed plants of certain wheat genotypes of the present study to accumulate or at least maintain the Cars pigments might be related to the proposed carotenogensis process. Despite the fact that photosynthetic pigments were affected in a wheat group-specific manner and F_v/Fm remained unaffected, gas exchange parameters of all wheat groups shared a common decreasing trend in response to salinity (Table 3). In the present study, 3–23% decreases in P_N were accompanied by 33–50% decreases in g_s, 20–35% decreases in C_i, and negligible modifications in F_v/Fm of the different wheat groups. The proportionality among these gas exchange attributes and RDM and SDM, i.e. in terms of salt-induced suppressions, suggests that photosynthetic production in these wheats has suffered more from stomatal and CO_2 diffusion barriers rather than biochemical malfunctioning. This proposal is supported by the observation that C_i of the salt-stressed plants of different wheat groups was decreased, in comparison to that of the non-stressed plants (Table 3). Accompaniment of decreases in P_N and C_i under stressful conditions has been

interpreted as lack of damage to the photosynthetic apparatus (Zhang et al. 2015). A further indication of lack of a serious salt-induced harm to the photosynthetic apparatus of the examined wheat groups is the fact that no notable modifications in F_v/Fm was observed. Partial stability of some of the spelt-macha (i.e. Chl and LPC) and emmer HW (i.e. Chl, LPC, SDM, and RDM) characteristics despite exposure to a severe level of water salinity in the present work was not far from expectation, as stability of spelt wheat chlorophyll index and grain yield attributes under unfavourable environmental conditions has been reported (Zuk-Golaszewska et al. 2015). While the general descending patterns of Na^+ accumulation and Na^+/K^+ ratios in the examined groups of wheat were as follows: emmer HW > durum FTW > spelt-macha HW > bread FTW (Table 4), and durum FTW > emmer HW > spelt-macha HW = bread FTW (Table 5), respectively, salt tolerance (i.e. assessed as percent decreases in SDM, RDM, and total plant dry mass due to salt exposure) followed the emmer HW > spelt HW = bread FTW > durum FTW pattern (Tables 3 and 4). Tetraploid wheats are, generally, expected to be more salt-sensitive, due mainly to the absence of D genome. But exceptions to this generalization are to be taken into account; in fact, landraces and endemic wheats are well-adapted to their environments and hence may indicate an appreciable level of tolerance to salinity (Badridze et al. 2009). Surprisingly, however, the Na^+/K^+ ratio was not found to be correlated with salt tolerance, at least in the studied emmer HW group of genotypes. Our results are apparently mismatched to those of Shavrukov et al. (2009), where they reported that salt exclusion is positively correlated to salt tolerance in wheats of different ploidy levels. However, a greater salt tolerance in the emmer HW landraces, compared to durum FTW examined in the present study is in line with the findings of Shavrukov et al. (2009). A plant species or genotype with low salt exclusion capability is considered salt-sensitive, unless maintains its dry mass or economic yield in the presence of potentially toxic concentrations of the salty ion. Accumulating a large amount of potentially harmful ions (e.g. Na^+) in plant tissues concomitant to a lack of damage symptoms (as it has been the case with the emmer wheats of the present study) might be an indication of co-occurring of Na^+-driven osmoregulatory measures and a strategy of ion exclusion (from entering sensitive cellular compartments) or its sequestration into vacuoles (Bendaly et al. 2016). As it is visualized in bi-plot (Fig. 1), the two emmer wheat genotypes along with the TRI 9652 durum wheat withstood 120 mM NaCl and this tolerance was highly correlated to the ionic attributes, in general, and Na^+/K^+ and Na^+ concentration, in particular. This inference was made as Na^+/K^+ and Na^+ concentration had positive loadings for PC1. The plot had the capacity to discriminate between tolerant and susceptible genotypes and interestingly the above-mentioned genotypes were clustered together across the wide range of NaCl concentrations attempted in this study. A better performance of the above-mentioned genotypes under the non-saline condition appeared, however, to be more closely correlated to plant dry mass attributes (i.e. SDM, RDM, V_{Root}), C_i, K^+, Chl, and Ca^{2+} concentration of the leaf, as these attributes had positive loadings on PC2. The Indian dwarf genotype examined in our work did not behave much different from the remaining hexaploid genotypes in reacting to water salinity, in spite of the fact that this wheat has been described as a drought-tolerant hexaploid wheat endemic to Indian subcontinent (Mori et al. 2013).

The importance of these results comes primarily from the relative accuracy of methods used and comprehensiveness of the set of traits examined in this study, which allowed us to more definitely understand the relationships among photosynthesis, osmolytes fluctuations, and plant dry mass production of ancient and modern wheats in a fairly wide range of irrigation water salinities. Evolution of polyploid wheats over the nearly 10 millennia-long course of cultivation is in fact a diversification continuum with wild emmer being at one end and bread wheat at the other (Matsuoka 2011). The novelty in our results is warranted to the finding that a greater absorption and, hence, tissue accumulation of the potentially hostile Na^+ ion is not detrimental to a set of emmer HW genetic resources (compared to durum FTW, spelt-macha HW, and bread FTW groups) that have been marginalized due to our negligence. We hope that our series of work will shed light on the potential of these valuable resources (i.e. as breeding materials) for combating concomitant climate change, drought and salinity episodes that are questioning crop production potential and food security worldwide.

Conclusions

Stress-induced physiological responses of the present emmer HW landraces and spelt-macha genotypes have not before been studied in depth. Differences in stomatal behaviour (reflected in g_s) and photosynthetic pigments

(displayed in Chl and Cars concentrations) seemed responsible for P_N and SDM differences of the salt-stricken plants from the non-stressed plants of all groups of wheat. While partial salt tolerance noted in bread FTW and spelt-macha hulled hexaploid wheats is attributed to genome D, concurrence of Na^+ absorption, transport and accumulation and plant dry mass sustainment under saline condition is a novel characteristic of the present underutilized emmer HW landraces. Salt tolerance, judged as salt-induced decreases in total plant dry mass followed the emmer HW > spelt HW = bread FTW > durum FTW descending pattern; our results uncovered that potency in salt tolerance due, perhaps, to Na^+ sequestration in the ancient tetraploid HW resources, i.e. emmer, is notably greater than the improved durum FTW ones. Although the picture for the presumed ion sequestration is not entirely clear, these ancestors of great value to our staple durum and bread FTW crops have the potential to assure future food security in the face of a challenging climate change. This work can be seen as a step forward towards anticipation of salinity tolerance of new durum FTW cultivars.

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Author contributions ZA and PE conceived and designed the experiment. ZA performed the experiment and analysed the data. PE wrote the manuscript and ZA provided editorial advice and prepared the tables.

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