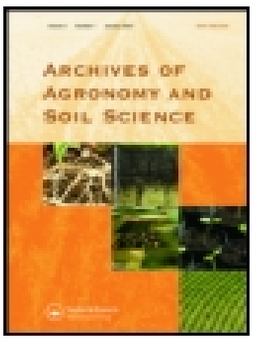

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Effects of drought stress on some agronomic and bio-physiological traits of *Trtiticum aestivum*, Triticale, and Tritipyrum genotypes

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ABSTRACT

Drought tolerance is a complex trait that involves different biochemical and physiological mechanisms in plants. It was the objective of the present study to evaluate the agronomic and biochemical responses of triticale, tritipyrum, and wheat to drought stress. For this purpose, twenty-seven genotypes were evaluated under two levels (non-stress and drought stress) of irrigation during 2015–2017. The metabolic traits studied included relative water content (RWC), membrane stability index (MSI), chlorophyll a (Chla), chlorophyll b (Chlb), carotenoids (Car), leaf proline content (Pro), leaf soluble carbohydrates (LSC), glycine betaine (GB), malondialdehyde (MDA), hydrogen peroxide (H_2O_2), seeds per spike (SS), seed weight (SW), biological yield (BY) and seed yield (SY). Drought stress increased Pro, LSC, and GB contents as well as lipid peroxidation through increasing MDA and H_2O_2 activities. However, both RWC and MSI indices as well as SS, SW, SY and BY reduced as a result of drought treatment although the least decrease of SY was observed at triticale group. During the two years of study, the tritipyrum genotypes exhibited their drought tolerance by accumulation of more LSC and GB as well as lower decrease in SW while the triticale ones responded by maintaining higher levels of RWC but producing less MDA and H_2O_2. It may, therefore, be concluded that the three species studied exploit different mechanisms to maintain tolerance against drought stress. Finally, correlation analysis indicated the positive effects of LSC on SY under both drought and normal conditions, which is obviously a promising trait in wheat, triticale, and tritipyrum that can be beneficially exploited in drought tolerance improvement programs.

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Bread wheat; biplot; drought tolerance; glycine betaine; malondialdehyde

Introduction

Bread wheat production, as the most important crop in the world, is being seriously affected by changes in global climate and precipitation patterns (Sayyah et al. 2015). Post-anthesis drought stress reduces grain yield in bread wheat due to impaired grain development associated with imbalanced levels of growth hormones (Sayyah et al. 2015; Abid et al. 2017), which may be the result of limited sink size, early senescence, lack of assimilate supply, and imbalanced levels of growth hormones involved in sink size development and grain filling (Yang et al. 2006; Abid et al. 2017). Thus, the high frequency of post-anthesis water deficits in cultivated regions make it essential to develop new drought tolerant cultivars of wheat. Decreased annual rainfall in arid

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regions requires the cultivation of drought tolerant genotypes or substitute cereals (Fang and Xiong 2015). The low within-species variability of wheat has led to proposals for improving drought tolerance in wheat through introgression from close relatives to the Triticeae species (Szira et al. 2008). Triticale (X. *Triticosecale* Wittmack) with a AABBRR genome is one of the most successful cereals, with superior resistance to both drought and cold conditions by maintaining the RR genome (Lelley 2006). Tritipyrum is a hybrid of durum wheat (2n = 4x = 28 AABB) and wheatgrass (2n = 2x = 14 E_bE_b), *Thinopyrum bessarabicum* (Lelley 2006). It could be used as a valuable source of genes for creating drought tolerance in wheat by wide-hybridization. All of the Triticeae species are closely related to bread wheat (*Triticum aestivum)* and can, thus, be used as a possible source of genetic improvement for drought tolerance.

Drought stress is a major environmental factor limiting plant growth and crop yield (Basu et al. 2016; Anjum et al. 2017). Drought-stricken lands have recently increased due to recent global climate change (Fang and Xiong 2015; Anjum et al. 2017). Plants adopt a variety of strategies to respond to drought stress; these include changes in plant biochemical and physiological processes, such as reducing water losses, enhancing antioxidant activity, and accumulating osmolytes (Praba et al. 2009; Almeselmani et al. 2012). Plant response to water stress depends on such factors as species, genotype, developmental stage, and stress severity and duration (Tester and Langridge 2010; Anjum et al. 2017). Selection of drought tolerant genotypes might be improved by exploiting new genetic resources that offer a high capacity for drought tolerance.

One consequence of stressful conditions in plants is the increase in cellular concentrations of reactive oxygen species (ROS), that naturally transform into hydrogen peroxide (H_2O_2), considered as both a signaling molecule and a regulator for the expression of certain genes in plant cells under drought stress (Hung et al. 2005). Cell membranes are one of the first targets of drought stress and the tolerance threshold of plants is determined by the maintenance of membrane integrity under drought conditions (Almeselmani et al. 2012). Malondialdehyde (MDA) is the final product of plant cell membrane peroxidation, serving as a marker of cell membrane injury (De Vos et al. 1991).

Plant adaptation to drought stress is associated with different metabolic adjustments leading to the accumulation of several organic solutes such as sugars (Hu et al. 2015), polyols, betaines (Khan et al. 2009), and proline (Giri 2011; Fang and Xiong 2015). Proline is known as a compatible solute and its accumulation is one of the most widespread plant responses to environmental stresses, especially that of drought (Verbruggen and Hermans 2008). It protects plant cells by scavenging reactive oxygen species (ROS) (Mittler et al. 2004; Hung et al. 2005), whereas carbohydrates are used to maintain plant metabolism and to save energy under drought (Khalid et al. 2010). Glycine betaine (GB) plays an important role as a compatible solute in plants under different environmental stresses (Wang et al. 2010; Giri 2011). Leaf soluble carbohydrates (LSC) are increased as reserves in the plant in response to drought stress and reduced soil water content (Zhang et al. 2014; Hu et al. 2015). The two basic functions attributed to these solutes (GB and LSC) are osmotic adjustment and cellular compatibility (Khan et al. 2009; Giri 2011).

No published report was found in the literature on the effects of drought stress on bio-physiological traits in Tritipyrum. Thus, the present study was designed to: 1) investigate the bio-physiological responses of Triticale and *Tritipyrum* species to drought stress as a tool for unveiling the basic mechanisms involved in their drought tolerance, and 2) explore the different biochemical and physiological responses to drought stress among wheat relative (Triticale and Tritipyrum) to exploit their superior genotypes to improve drought tolerance in wheat through hybridization.

Materials and methods

Plant material and growth conditions

The material used for the experiments consisted of 27 different genotypes from three different species from the Triticeae tribe including: 1) Tritipyrum lines (including primary hexaploid tritipyrum

lines Ka/b, St/b, La/b, Cr/b, La(4B.4D)/b (a substitution line) and Az/b, and hybrids between parental genotypes in different breeding generations including $KaCr_3$, $MaCr_3$ $KaCr_4$, $StCr_4$, $MaCr_4$ and $KaCr_6$; 2) Triticale lines (T_{4103}, T_{4108}, T_{4116}, T_{4115}, and M45); and 3) hexaploid *Trtiticum aestivum* L. lines of Iranian origin (Roshan (drought tolerant), Omid, Alvand, Double haploid (DH) (originated from Kavir × Bam cross), M_{757}, Kavir, Bam, Baft, and Niknejad). The primary parental tritipyrum seeds were obtained from the John Innes Center (United Kingdom), but the seeds of different generations were obtained through hybridization and production of advanced generations. Triticale seeds and bread wheat genotypes were obtained randomly from the Dryland Agricultural Research Institute of Maragheh, Iran. Seeds were hand-planted in rows 3 m long spaced 20 cm from each other (0.4 m × 3 m) on 24 October 2015 and 2 November 2016 at the Lavark Research Farm of Isfahan University of Technology located 40 Km southwest of Isfahan (32° 32′N, 51° 23′ E, 1630 m asl), Iran. Also, all the genotypes harvested at 7 July at both years of study. The soil at this site was silty clay loam with pH 7.3–7.8 and a bulk density of 1.3 g cm^{-3} in the top 50 cm. It had an organic C content of 2.7 g Kg^{-1} and a water-holding capacity of 240 g kg^{-1} at field capacity (determined gravimetrically). The monthly mean of rainfall and temperature data averaged at both years is presented in Figure S1.

Experimental design and irrigation treatments

The experiment was carried out as a split plot design based on a randomized complete block design with three replications in each of two years (2015–2016 and 2016–2017). The irrigation treatments were considered as the main plots and entries (genotypes within species) as the subplots. In each subplot, the genotypes were sown in three rows each 3 m long and spaced 20 cm from each other (0.4 m × 3 m). Normal agronomic practices (weed and disease control) were performed during the experiments. Surface application of 120 (Kg ha^{-1}) N and 20 (Kg ha^{-1}) P was carried out during the experiment at both year of study and 60 (Kg ha^{-1}) N during the spring stem elongation. Water stress was applied during the growing season from anthesis to the seed filling stage. Monthly mean temperature during different seasons averaged over 2015–2017 is presented in Figure S1. The meteorological data indicated no rain events during the drought stress treatment (from 1st April to 30th June during the years 2015 and 2016) (Figure S2). Water was delivered from a pumping station via polyethylene pipes and the water volumes applied were measured using a volumetric counter. In the normal and drought environments, respectively, plants were irrigated when 50% and 75% of the total available soil water was depleted from the root zone, respectively. The equation

$$I = [(\theta_{FC} - \theta_i)/100]\ D \times B) \tag{1}$$

was used for the determination of irrigation depth, where I is irrigation depth (cm), θ_{FC} (–0.03 MPa) is soil gravimetric moisture percentage at field capacity (22%), θ_i (–1.5 MPa) is soil gravimetric moisture percentage at irrigation time (10%), D is root-zone depth (60 cm), and B is soil bulk density in the root zone (1.3 g cm^{-3}) (Clarke et al. 2008). The total amounts of the applied irrigation water were 4773.2 and 3351 m^3 ha^{-1} in 2015 and 4250.3 and 2920 m^3 ha^{-1} in 2016 under normal and drought stress conditions, respectively.For physiological and biochemical traits, five mature leaves below the flag leaf were sampled at the grain filling stage in the morning for all the genotypes and traits (Praba et al. 2009).

Measurement of traits

Physiological traits

Relative water content (RWC) was calculated according to the following formula due to Ritchie and Nguyen (1990):

$$\text{RWC}\,(\%) = [(\text{fresh weight} - \text{dry weight})/(\text{turgid weight} - \text{dry weight})] \times 100 \tag{2}$$

Membrane stability index

Membrane stability index (MSI) was calculated using the formulae

$$(\mathrm{MSI} = [1-(C_1/C_2)] \times 100) \quad (3)$$

(Praba et al. 2009). First leaf conductivity (C_1) was calculated by placing 500 mg of fresh leaf sample washed in deionized water (20 ml) and incubating in the dark at room temperature for 24 h. The samples were transferred to a water bath at 80°C and then the second conductivity (C_2) reading was recorded.

Photosynthetic pigments

The photosynthetic pigments were measured using 25 (mg) of fresh leaf. The fresh leaf samples were homogenized in the dark in the presence of 2 ml of 80% acetone in the presence of 0.1 g $CaCO_3$ and the homogenate was centrifuged at 5,000 × *g* for 10 min at 5 °C. The supernatant was removed and the chlorophyll fractions (chlorophyll a, chlorophyll b) and carotenoids (Car) were quantified using a spectrophotometer to measure their absorbance at 470, 646.8, and 663.2 nm. The concentrations of Chla, Chlb and Car were obtained according to Lichtenthaler and Buschmann (2001).

Proline assay

Leaf proline (Pro) was measured using the method of Bates (1973). Briefly, 500 mg of fresh leaves was ground in 10 ml of 3% sulfosalicylic acid in aqueous solution and the extract was filtered. Then, 2 ml of ninhydrin reagent and 2 ml of glacial acetic acid were added to 2 ml of the extract. A water bath (100°C) was used to boil the reaction mixture for one hour. The mixture was cooled on ice before 4 ml of toluene was added and mixed. The toluene phase was separated and its absorbance was measured at 520 nm against the toluene blank using a spectrophotometer (UNICO Model UV-2100).

Glycine betaine

Glycine betaine (GB; Sigma Inc.) was used as a standard. Determination of the GB in the samples was carried out using 25 mg of the dried and ground leaf incubated with 2 ml of distilled water (Grieve and Grattan 1983). The homogenized mixture was then continuously shaken for 4h at 25°C, then centrifuged at 10,000 × *g* for 10 min at 25°C before the supernatant was separated. Finally, quantification of GB was carried out at 365 nm using a spectrophotometer.

H_2O_2 assay

H_2O_2 content was estimated according to Loreto and Velikova (2001). For this purpose, a standard calibration curve was prepared using different H_2O_2 concentrations. Briefly, 5 ml of 0.1% (w/v) trichloroacetic acid (TCA) was added to 0.07 (g) of fresh leaf tissue and homogenized. The homogenate was then centrifuged at 12,000 × *g* for 15 min before 0.5 ml of the supernatant was added to 0.5 ml of potassium phosphate (10 mm) buffer (pH 7.0) and 1 ml of KI (1 M). The absorbance of the supernatant was measured at 390 nm and the H_2O_2 content was calculated by comparison with a standard calibration curve.

Malondialdehyde

Lipid peroxidation was investigated by measuring malondialdehyde (MDA) content (De Vos et al. 1991). First, 0.2 g of the frozen fresh leaf sample was homogenized in 3 mL of TCA solution (10% w/v) and the aliquots of the filtrates were heated in 0.25% thio barbituric acid for 30 min. Then, a final cooling was carried out in an ice bath. The absorbance of the solution was recorded at 532 nm followed by correlation for the nonspecific absorbance at 600 nm. The amount of MDA was determined based on an extinction coefficient of 155 mM^{-1} cm^{-1}.

Leaf soluble carbohydrates

Leaf soluble carbohydrates (LSC) in leaf were quantified using the method of Dubois et al. (1956).

First, 1 (g) of dried leaves were mixed with 15 (ml) of ethanol (80%). Then, 1 ml of aliquot was mixed with 1ml of 5% phenol (w/v) and 5 ml of concentrated H_2SO_4. The mixture was shaken and placed in a water bath at 25°C for 30 min. The absorbance was determined at 490 nm using a spectrophotometer. Glucose (Sigma, Inc) was used as the standard for measuring LSC.

Agronomic traits

Five random plants were harvested from each row and the average seed yield (g) (SY), seeds per spike (SS), 1000- seed weight (SW) and biologic yield (t ha^{-1}) (BY) was recorded based in a 15 plant for each genotype in each plot.

Statistical analysis

The data were subjected to combined analysis of variances using PROC GLM of SAS version 9.3 (SAS Institute 2011). Means comparisons were conducted using the least significant differences (LSD) test. Principal component analysis (PCA) was employed to identify the interrelationships among the studied genotypes and all the measured traits. The correlation coefficients between the traits were calculated using the proc CORR of SAS. The biplot was drawn using the Stat Graphics software.

Results and discussion

Changes in biochemical, physiological and agronomical characteristics

Investigation of between- and within-species variations for different traits (physio-biochemical and seed yield under drought stress and non- stress conditions is useful for identification of new genetic sources of drought tolerance traits (Fleury et al. 2010). The present research investigated plant responses treatment, as reflected in, biochemical and agro-physiological traits and seed yield to environment (year), drought stress (as treatment), and genetic (species and genotypes) variables (Table 1).

The analysis of variance showed highly significant ($P < 0.01$) differences between drought stress and non-stress treatments for all the studied traits, except Car concentration (Table 1). The effect of year was also significant for all the traits, except Car concentration and SY ($P < 0.01$) (Table 1). Different species and within-species genotypes showed significant variation for all the studied traits (Table 1).

The significant interaction effects of year × species on LSC, Pro, GB, and seed yield demonstrated that the environmental condition (as year) influenced these traits differently in these species (Table 1). The significant interaction effects of species × treatment on MSI, Chla, Chlb, Car, Pro, LSC, GB, SS, SW and SY similarly demonstrated that the response to drought exposed by these traits differed between species (Table 1). The data thus obtained might be helpful in understanding the differing drought tolerance mechanisms in members of the *Triticeae* family, since there is a high level of synteny among the chromosomes throughout the *Triticeae* family (Szira et al. 2008). The treatment × genotype (species) interaction effect was significant for all traits, except MSI (Table 1).

Comparisons of means of all the studied traits under the two (non-stress and drought stress) treatments are reported in Table 2. Water stress reduced RWC, MSI, SS, SW, BY and SY in both years, while the other traits including Car, Chla, Chlb, Pro, LSC, and GB increased in the drought-stress conditions relative to those of the control (Table 2). RWC showed a significant decrease from the non-stress to the drought-stress conditions for wheat and triticale groups in both years (Table 2). The highest reduction (12.3%) in RWC was observed in wheat groups grown in 2015 (Table 2), whereas the least reduction (2.9%) was observed in the tritipyrum group grown in 2016 (Table 2).

Table 1. Effects of year, drought stress, species, genotype, and their interactions on the traits studied.

		Mean squares												
Source of variation	DF	RWC	MSI	H_2O_2	MDA	Chla	Chlb	Car	Pro	LSC	GB	SS	SW	SY	BY
Year	1	**	**	**	**	**	**	ns	**	**	**	**	**	ns	**
Replication (year)	4	-	-	-	-	-	-	-	-	-	-	-	**	-	*
Treatment	1	**	**	**	**	**	**	ns	**	**	**	**	**	**	**
Treatment × year	1	ns	ns	ns	ns	ns	ns	ns	ns	**	ns	ns	**	ns	ns
Replication (treatment × year)	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Species	2	**	ns	**	**	**	**	**	**	**	**	**	**	**	**
Genotype (species)	24	**	**	**	**	**	**	**	**	**	**	**	**	**	**
Species × year	2	ns	ns	ns	ns	ns	ns	ns	**	**	**	ns	ns	**	ns
Species × treatment	2	ns	**	ns	ns	**	**	**	**	**	**	**	**	*	ns
Species × treatment × year	2	ns	ns	ns	ns	ns	ns	ns	ns	ns	**	ns	ns	ns	ns
Year × genotype (species)	24	ns	ns	ns	ns	ns	ns	ns	ns	**	**	ns	ns	ns	ns
Treatment × genotype (species)	24	**	ns	**	**	**	**	**	**	**	**	**	**	ns	*
Genotype × treatment × genotype (species)	24	ns	ns	ns	ns	ns	ns	ns	ns	**	ns	ns	ns	ns	ns
Residual	210	74.79	51.24	0.00006	0.025	0.049	0.021	0.008	12.52	1.38	0.035	12.70	11.81	7.95	24.13

*and ** are significant at $P < 0.05$ and $P < 0.01$, respectively. RWC: Relative water content; MSI: Membrane stability index; hydrogen peroxide: H_2O_2; MDA: Malondialdehyde; Chla: Chlorophyll a; Chlb: Chlorophyll b; Car: Carotenoid; Pro: Proline; LSC: Leaf Soluble Carbohydrates; GB: Glycine betaine, SS: Seeds per spike; SW: 1000- seed weight; SY: Seed yield/plant; BY: Biological yield.

Table 2. Physio-biochemical characteristics and seed yield of bread wheat, triticale, and tritipyrum grown under the two (control and drought) irrigation regimes in two years of study (2015 and 2016).

		2015			2016			
Trait	Species	Control	Drought	Difference	Control	Drought	Difference	Mean
	wheat	63.2	55.4	**	69.68	62	*	62.57
RWC (%)	triticale	72.29	66.68	**	78.82	71.91	**	72.42
	tritipyrum	70.46	63.16	*	76.04	68.91	ns	68.89
	wheat	82.39	76.66	*	87.16	81.52	*	81.93
MSI (%)	triticale	83.36	75.58	**	87.82	80.15	**	81.72
	tritipyrum	85.07	72.97	**	89.01	76.95	**	80.99
	wheat	0.023	0.026	ns	0.032	0.044	**	0.031
H_2O_2 (mmol g^{-1} FW)	triticale	0.024	0.027	ns	0.016	0.031	ns	0.024
	tritipyrum	0.021	0.025	ns	0.016	0.017	ns	0.020
	wheat	1.09	1.26	**	1.29	1.46	**	1.27
MDA (µg g^{-1} FW)	triticale	1.06	1.26	**	1.25	1.49	**	1.25
	tritipyrum	0.96	1.15	ns	0.98	1.22	**	1.07
	wheat	1.50	1.66	*	1.30	1.47	**	1.47
Chla (mg g^{-1} FW)	triticale	1.70	1.84	**	1.49	1.62	**	1.66
	tritipyrum	1.79	1.76	ns	1.60	1.56	ns	1.68
	wheat	0.53	0.62	*	0.47	0.56	*	0.54
Chlb (mg g^{-1} FW)	triticale	0.62	0.77	**	0.58	0.71	**	0.67
	tritipyrum	0.72	0.66	ns	0.68	0.60	ns	0.66
	wheat	0.54	0.64	**	0.56	0.62	**	0.58
Car (mg g^{-1} FW)	triticale	0.68	0.74	**	0.67	0.77	**	0.71
	tritipyrum	0.72	0.68	ns	0.70	0.65	ns	0.69
	wheat	13.55	27.15	**	15.57	28.83	**	21.27
Pro (µmol g^{-1} FW)	triticale	12.57	25.33	**	13.04	26.06	**	19.25
	tritipyrum	15.72	25.83	**	19.83	30.96	**	23.08
	wheat	10.22	13.66	**	9.64	10.65	**	11.05
LSC (mg g^{-1} DW)	triticale	9.38	13.04	**	8.77	10.16	**	10.34
	tritipyrum	13.19	18.08	**	15.02	18.92	**	16.30
	wheat	3.06	3.27	*	2.46	2.75	*	2.88
GB (µ mol g^{-1} DW)	triticale	3.23	3.64	*	3.50	3.73	ns	3.52
	tritipyrum	3.33	3.87	*	2.39	3.17	**	3.19
	wheat	36.02	31.62	**	35.88	28.13	**	32.91
SS	triticale	30.58	29.80	n.s	30.17	27.97	n.s	29.63
	tritipyrum	47.51	42.78	**	46.55	39.03	**	43.96
	wheat	27.15	21.12	**	25.77	18.34	**	23.14
SW (g)	triticale	18.89	14.53	**	17.83	12.82	**	16.01
	tritipyrum	29.21	25.22	*	28.30	22.50	*	26.30
SY (g $plant^{-1}$)	wheat	18.8	14.1	**	17.31	14.12	**	15.83
	triticale	19.4	15.31	**	20.8	17.42	**	18.48
	tritipyrum	13.51	9.83	**	15.68	12.81	**	12.96
BY (t ha^{-1})	wheat	27.15	21.12	**	25.77	18.54	**	23.14
	triticale	18.89	14.53	**	17.83	12.82	**	16.01
	tritipyrum	29.21	25.22	ns	28.29	22.50	*	26.30

**and ** are significant at $P < 0.05$ and $P < 0.01$, respectively. ns non-significant . RWC: Relative water content; MSI: Membrane stability index; H_2O_2: Hydrogen peroxide; MDA: Malon dialdehyde; Chla: Chlorophyll a; Chlb: Chlorophyll b; Car: Carotenoid; Pro: Proline; LSC: Leaf Soluble Carbohydrates; GB: Glycine betaine; SS: Seeds per spike; SW: 1000- seed weight; SY: Seed yield/ plant .; BY: Biological yield.

Mean comparison of different traits under different treatments of irrigation

Physiological traits

A decrease in membrane stability under drought stress reflects the extent of lipid peroxidation caused by reactive oxygen species (ROS) (Sanchez-Rodriguez et al. 2010). Leaf RWC widely used as an indicator of tolerance to drought stress (Sanchez-Rodriguez et al. 2010). RWC reduced significantly from the control to drought stress in both years for wheat and triticale species (Table 2). The highest RWC was observed in triticale group in both years of study under drought treatment (Table 2). MSI reduced significantly from the control to drought stress in both years for all the species investigated (Table 2). In 2015, the highest values of MSI under drought stress were

obtained with the triticale group (Table 3), but wheat group contained less MSI than the triticale as confirmed by the higher MDA and H_2O_2 concentrations in wheat genotypes (Table 2).

Biochemical traits

H_2O_2 concentration increased significantly in the wheat group grown in 2016 from 0.032 mmol g^{-1} FW under the control conditions to 0.044 mmol g^{-1} FW in drought stress conditions (Table 2). The lack of any detectable significant change in the H_2O_2 activity of the other two species could be due to the relatively smaller adverse effect of drought stress on the lipid peroxidation of triticale and tritipyrum species; however, the least increase in H_2O_2 content under drought stress was recorded for the triticale groups in both years (Table 2).

The lipid peroxidation index also increased in both years as a result of increase in MDA activities under drought stress, except for the tritipyrum group in 2015 (Table 2). The highest MDA level (1.49 µg g^{-1} FW) was recorded in wheat under stress conditions in 2016, whereas the least (0.96 µg g^{-1} FW) belonged to the tritipyrum group under the control conditions in 2015 (Table 3). The highest increases in MDA were obtained with the tritipyrum group in 2015 (19%) and 2016 (24%), respectively (Table 2). This indicated the sharp response of the tritipyrum genome to peroxidation activity under water deficit.

The triticale group exhibited lower increases in MDA activities than the other ones (Table 2), which indicates a higher anti-oxidative ability of it, which, in turn, reflects a higher drought tolerance.

The increase in MDA, as a product of membrane system injury, has been reported in tomato (Sanchez-Rodriguez et al. 2010) and bread wheat (Wang et al. 2010; Chakraborty and Pradhan 2012; Bouchemal et al. 2017) under drought stress.

The ability to maintain the functionality of the photosynthetic machinery under stress is of major importance to drought tolerance (Loreto and Velikova 2001). Photosynthetic adaptation of plants to drought stress involves a complex interaction of hormones, ROS, and other metabolic events at different plant growth stages (Loreto and Velikova 2001; Almeselmani et al. 2012; Basu et al. 2016).

Comparison of means showed that chlorophyll (a and b) content increased significantly under drought stress in wheat and triticale genotypes in both study years, but the decrease in chlorophyll (a and b) in the tritipyrum group was not significant (Table 3). The greatest drought- induced increases in Chla (13%) and chlb (22%) were obtained with wheat and triticale groups grown in 2016, respectively (Table 2).

High chlorophyll concentration under drought stress is a desirable trait because it indicates a low degree of phyto inhibition of the photosynthetic system (Askari and Ehsanzadeh 2015). In this study, the reductions in Chla and Chlb in the tritypyrum group under drought could be due to their faster breakdown or dissociation in that species or, alternatively, to changes in the pigment -protein complexes (Fang and Xiong 2015). The significant increase in the chlorophyll (a and b) concentration of wheat and triticale groups under drought stress (Table 2) could be the result of declining cellular growth relative to chlorophyll synthesis (Garcıa-Valenzuela et al. 2005). In contrast to out finding, Chakraborty and Pradhan (2012) and Bouchemal et al. (2017) reported decreased chlorophyll content in bread wheat under drought stress. These discrepancies in the results could be the results of differences in drought intensity, stress duration, and or genotypes studied (Bouchemal et al. 2017).

The enhanced photoprotective capacity of the leaves is supported by the antioxidative function of carotenoid under drought stress (Bhargava and Sawnat 2013; Askari and Ehsanzadeh 2015).

In this study, wheat and triticale groups showed an increase in carotenoids content under drought, but carotenoid content was observed to decline in tritipyrum genotypes (Table 2), implying that changes of carotenoid content are mostly species-dependent, which as also reported by Bhargava and Sawnat (2013).

The highest carotenoid content (0.74 mg g^{-1} FW) was recorded for the triticale group in 2016 under drought conditions, whereas the least (0.54 mg g^{-1} FW) was observed in the wheat group under control conditions in 2015 (Table 2). *Foeniculum vulgare* has been pointed to exhibit an

Table 3. Correlation coefficients of different traits under stress (above diagonal) and non-stress (below diagonal) conditions averaged over the two study years (2015 and 2016).

Traits	RWC	MSI	H_2O_2	MDA	Chla	Chlb	Car	Pro	LSC	GB	SS	SW	SY	BY
RWC	1	0.27	−0.024	−0.34*	0.19	0.25	0.23	0.18	0.25	0.21	−0.13	0.09	0.05	−0.02
MSI	0.14	1	0.26	−0.14	−0.06	−0.01	−0.04	0.26	−0.002	0.04	−0.07	−0.3	0.15	−0.008
H_2O_2	−0.05	−0.01	1	0.47**	0.15	0.23	0.13	−0.46**	−0.77**	−0.32*	−0.33*	−0.45**	−0.35*	−0.31
MDA	−0.16	0.25	0.41*	1	0.027	0.004	0.13	−0.46**	−0.62**	−0.52**	−0.4*	−0.42**	−0.18	−0.30
Chla	0.43**	0.01	−0.29	−0.14	1	0.15	0.91**	0.30	−0.18	−0.32*	0.06	−0.02	−0.17	−0.16
Chlb	0.39*	0.016	−0.34	−0.12	0.96**	1	0.86**	−0.27	−0.18	−0.13	−0.02	−0.08	−0.19	−0.22
Car	0.43**	−0.08	−0.39*	−0.15	0.97**	0.96**	1	−0.28	−0.15	−0.25	−0.1	−0.11	0.34*	−0.27
Pro	−0.034	−0.25	−0.28	−0.62**	0.04	0.01	−0.01	1	0.63**	0.40*	0.08	0.06	0.079	0.19
LSC	−0.16	0.25	−0.55**	−0.61**	0.11	0.14	0.11	0.59**	1	0.43**	0.3	0.56**	0.54**	0.46**
GB	0.03	0.20	−0.45**	−0.55**	0.17	0.14	0.21	0.48**	0.54**	1	0.06	0.27	0.09	0.11
SS	−0.15	0.05	−0.16	−0.17	−0.04	−0.02	−0. 15	0.08	0.4*	0.18	1	0.48**	0.58**	0.50**
SW	−0.08	0.05	−0.21	−0.36*	0.05	0.08	0.08	0.29	0.62**	0.42*	0.58**	1	0.44*	0.53**
SY	0.27	0.22	−0.11	−0.22	−0.07	−0.05	0.11	0.24	0.56**	0.13	0.61**	0.6**	1	0.42**
BY	−0.11	−0.07	−0.16	−0.06	−0.07	−0.02	−0.09	−0.006	0.32*	0.11	0.57**	0.54**	0.41**	1

*and ** are significant at $P < 0.05$ and $P < 0.01$, respectively. RWC: Relative water content; MSI: Membrane stability Index; H_2O_2: hydrogen peroxide, MDA: Malon dialdehyde; Chla: Chlorophyll a; Chlb: Chlorophyll b; Car: Carotenoid; Pro: Proline; LSC: Leaf Soluble Carbohydrates; GB: Glycine betaine; SS: Seeds per spike; SW: 1000- seed weight; SY: Seed yield/plant.; BY: Biological yield.

increased carotenoid content under drought stress (Askari and Ehsanzadeh 2015), which is contrary to observations in bread wheat (Chakraborty and Pradhan 2012).

Leaf proline content increased significantly from the control to the drought stress conditions in all the species both in 2015 and 2016 (Table 2). Accumulation of Pro as a clear marker of drought stress could be due to the induction of its biosynthesis and/or its oxidation inhibition under water stress (Verbruggen and Hermans 2008). The highest Pro content (30.96 μmol g^{-1} FW) was observed in the tritipyrum group under drought stress in 2016, whereas the triticale group recorded the lowest (12.57 μmol g^{-1} FW) under the control conditions in 2015 (Table 2). The range of Pro accumulation was very wide among the species investigated from normal to drought treatment (Table 2). The highest increase (100%) was recorded with the triticale group from control (12.57 μmol g^{-1} FW) to 25.33 (μmolg^{-1} FW) under drought treatment in 2015, whereas the least increase (56%) was observed in the tritipyrum group in 2016 (Table 2). An increase in the leaf Pro content of bread wheat has been reported under drought stress (Chakraborty and Pradhan 2012). Moreover, recent breeding efforts have shown that some tritipyrum genotypes in this study are able to accumulate more Pro than the wheat species grown in affected by drought stress.

The variation in LSC concentration has been mainly attributed to variations in major soluble carbohydrates (i.e., sucrose and hexose) in different genotypes and species (Ruuska et al. 2006; Hu et al. 2015). The LSC of the studied genotypes increased significantly from the control to the drought stress treatments in all three species (Table 2). The highest (18.92 mg g^{-1} DW) and the lowest (8.77 mg g^{-1} DW) LSCs concentrations were observed in the tritipyrum and triticale under drought and control conditions, respectively, in 2016 (Table 2). The greatest (39%) increase in LSC under drought stress belonged to the triticale group in 2015 (Table 2). This is confirmed by similar studies that reported increasing LSC in wheat leaves under drought stress (Ruuska et al. 2006; Zhang et al. 2014; Hu et al. 2015). The tritipyrum genotypes showed higher intrinsic levels of LSC (Table 2), indicating the high osmotic adjustment of tritipyrum genotypes via LSC under drought stress.

The genotypes known for their natural GB accumulation have also been characterized by the ability to grow well in drought and saline environments (Giri 2011). All the species investigated, except for the triticale group, exhibited significant increases in their mean values of GB under the drought stress treatment in 2016 (Table 2). In both years, the highest content for GB increase belonged to the tritipyrum group under drought conditions, but the lowest GB level belonged to the wheat group under normal conditions (Table 2). The higher accumulation in GB in the tritipyrum genotypes under drought stress could be due to important role of this metabolite in this genome to respond biochemically to drought stress (Table 2).

Agronomical traits

Water deficit substantially decreased SS, SW, SY and BY in all the genotypes within the species investigated (Table 2). The least reduction in SS was measured in 2015 (2.5%) and 2016 (7.2%) in the triticale group (Table 2), whereas the highest seed yield reduction (21.6%) was observed in wheat group in 2016 (Table 2). The highest reduction in SW (28%) was observed in wheat group in 2016, but the least reduction was observed in tritipyrum (13.6%) in 2015 (Table 2). The least reduction in seed yield was measured in 2015 (21%) and 2016 (16%) in the triticale group (Table 2), whereas the highest seed yield reduction (27%) was observed in tritipyrum in 2015. The highest reduction in biologic yield (55%) was observed in wheat in 2015, but the least reduction (13%) was observed in tritipyrum group in 2015 (Table 2).

The comparison between three different species

Wheat genotypes exhibit lower biochemical responses to drought stress (Table 2). It could be suggested that the responses to drought stress in the wheat group, compared with other species, were triggered by their higher MSI (Table 2), which is consistent with the report by Wang et al. (2010). This leads to the conclusion that the biochemical basis of drought tolerance in triticale

genotypes is associated with higher MSI, higher water retention capacity (RWC), and low MDA and H_2O_2 concentrations under water deficit conditions. This suggests the importance of osmotic adjustment in the reduction of lipid peroxidation injuries in the triticale genome under drought conditions. Also, it could be suggested that the drought tolerance mechanism in the tritipyrum genotypes is related to their high accumulation of Pro, GB, and LSC. This hypothesis suggests the involvement of non-enzymatic antioxidant systems, such as osmotic adjustment through carbohydrate accumulation, in tritipyrum drought tolerance.

Within-species genotype discrimination

Comparison of means revealed that RWC (under both treatments) and MSI (under drought treatment) recorded their highest values with T_{4116} in the triticale group (Table S1). The least H_2O_2 activity (0.015 mmol g^{-1} FW) under normal treatment was observed in T_{4116} while $KaCr_3$ and $KaCr_6$ genotypes from the tritipyrum group exhibited the least activity (0.019 mmol g^{-1} FW) under drought treatment (Table 3). The least MDA value under both control and drought treatments were obtained with T_{4116} genotype (Table S1). The highest Chla and Chlb values in both (normal and drought) treatments were denoted to St/b genotype from the tritipyrum group (Table S1). Finally, the highest carotenoid level (0.79 mg g^{-1} FW) under drought stress was recorded in T_{4103} (Table S1).

The biplot graph discriminated the distributions of different traits and genotypes among the species based on principal component analysis (PCA) (Figure 1). Under drought conditions, the highest values of Pro, GB, and LSC were observed in $KaCr_3$ and Az/b (Figure 1(b)) while the highest SS, SW, and BY values were obtained in T_{4115} (in the triticale group) (Figure 1(b)). The genotypes exhibiting SY potential under non-stress conditions (Figure 1(a)) showed superiority under drought stress (Figure 1(a)). Nevertheless, T_{4116} was identified as a superior triticale genotype that could produce the highest SY and BY under both normal and drought stress conditions (Figure 1). T_{4108}, T_{4115}, M_{45}, and Roshan followed T4116 in SY potential under drought stress conditions (Figure 1(b)).

Trait correlations

In order to determine the relationships among the traits potentially useful in drought tolerance breeding programs, the correlation coefficients between the measured traits were calculated. According to Table 3, statistically significant negative correlations among H_2O_2 and MDA traits,

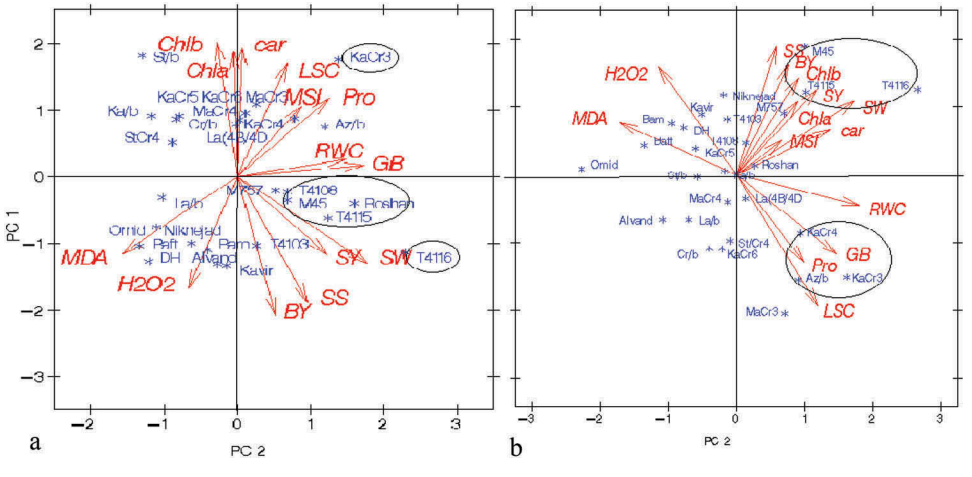

Figure 1. The biplot display for different traits and the within-species genotypes evaluated under drought stress (a) and non-stress conditions (b) averaged over two years.

with Pro, LSC and GB were detected under drought stress conditions averaged in both years (Table 3). On the other hand, the lack of correlation between both physiological traits including RWC and MSI with biochemical ones (Pro, LSC and GB) indicates that each trait is a potential indicator of different biological responses to drought (Table 3). Moreover, a positive and significant correlation was observed among Pro, GB, and LSC under both drought and control treatments. A significant and negative correlation (– 0.34**) was found between RWC and MDA (Table 3) under drought stress. Reduction in RWC under drought was highly correlated with increase in MDA content, suggesting a relationship with cleavage of the membrane (Table 3), which is consistent with the findings of Almeselmani et al. (2012) and Basu et al. (2016). In the present study, however, physiologic traits (RWC and MSI) were not correlated with drought resistance as expressed by not significant correlation with seed yield, SS, SW and BY under drought conditions; hence, RWC and MSI could not be employed as suitable physiologic indicators to select superior drought tolerant cultivars in these genotypes. The greater increase in LSC under drought stress in all species could be due to the enhanced rate of hydrolysis of complex carbohydrates storage and/or transfer them within the leaf as a result of reduced water potential (Zhang et al. 2014; Hu et al. 2015). This supplementary function of LSC showed that significant correlation of LSC with SS, SW and SY under drought stress in all the species studied (Table 3). Generally, LSC and Car (non-enzymatic antioxidants) had positive significant correlation under drought stress with SY in the species investigated (Table 3). These two traits could, therefore, be employed for seed yield improvement in the studied species under drought conditions.

On the other hand, SY showed significant and negative correlations with H_2O_2 (–0.35**) and significant and positive correlation with Car (0.34**), LSC (0.54**), SS (0.58**), seed weight (0.44**) and BY (0.42**) under the drought stress treatment. The non- significant correlation detected between Pro and SY (Table 3) demonstrated no critical effect of this osmolite on improving SY under drought stress. The highest SY in triticale under drought stress, suggests the existence of a more efficient antioxidant system for removing H_2O_2 (Table 3) and a better conservative cell membrane mechanism in the triticale genome under drought stress. It may, therefore, be inferred that those cellular processes that increase proline and LSC content also seem to be more effective in decreasing H_2O_2 and MDA production, thereby reducing plant cell damages under water stress (Table 3).

Conclusion

The high seed yield in superior genotypes of tritipyrum and triticale promises new paths for further molecular investigations of drought stress tolerance via osmolite accumulation. The superior triticale genotypes T_{4116}, T_{4115}, T_{4108} and the superior tritipyrum genotypes $KaCr_3$ and $MaCr_3$ are recommended as valuable gene reservoirs for use in hybridization programs to achieve greater drought tolerance in wheat germplasm, or to obtain segregating populations for further genetic studies on drought tolerance breeding under rainfed conditions or cultivation in semi- arid climates.

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