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PERFORMANCE OF DIVERSE WHEAT GENETIC STOCKS UNDER MOISTURE STRESS CONDITION

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Abstract

This study was conducted to evaluate divergent wheat germplasm for their performance under drought and control conditions. The germplasm consists of wheat land races of Pakistan, advanced D-genome synthetic derivatives and high yielding varieties of Pakistan. This wide array of germplasm was selected to identify sources, which can be opted later by the wheat breeders while breeding for drought tolerance. The evaluation parameters involved some important physiochemical testing and morphological characteristics in the field under drought and control conditions. Based on these parameters, 13 wheat genotypes were selected on the basis of their best performance regarding morphological and physiological parameters. These genotypes exhibited higher yield under drought stress conditions and increased percentage of proline, sugar, SOD and protein content under laboratory conditions as compared to the susceptible genotypes. Correlation studies revealed that grains per spike (GPS) and thousand grain weight (TGW) had direct relationship with spike length (SL), proline and sugar content under both control and drought conditions. Thus, these parameters can be used as selection criteria for the identification of tolerant genotypes.

Key words: Breadwheat, Drought, Landraces, Synthetic hexaploid, Yield, Abiotic stress.

Introduction

Wheat productivity mainly depends on high yielding varieties having tolerance to various biotic and abiotic stresses. Due to modern agricultural systems, newly bred cultivars possess very little potential to cope with abiotic stresses due to their narrow genetic base, which poses a threat to global food security (Newton *et al*., 2010). The landraces of wheat provide a broad genetic basis for improving various characteristics in modern cultivars (Dotlacil *et al*., 2010) to show better adaptation to water stress. Similarly, genetic diversity can also be increased through interspecific hybridization products, commonly known as synthetic hexaploids, produced through crosses between *Triticum turgidum* and *Ae. tauschii* accessions (2n=6x=42, AABBDD). These interspecific crosses are being further exploited to extend the narrow genetic makeup of breadwheat cultivars. Many agronomically important traits present in the wild grass progenitor of wheat (*Ae. tauschii*, 2n=2x=14, DD) have been transferred into wheat through this route (Villareal & Mujeeb-Kazi, 2003).

A number of morphological and physiological traits associated with drought tolerance have helped in the identification of drought tolerant genotypes under field conditions (Ahmad *et al*., 2000; Bibi *et al*., 2013; Razzak *et al.,* 2013). Several *in vitro* studies have also been identified using drought-inducing chemicals such as polythethylene glycol (PEG) for identifying the drought tolerance (Galovic *et al*., 2005; Khan *et al.,* 2012; Nawaz *et al*., 2013). Tatar & Gevrek (2008) have proved that proline and lipid peroxidation content increase under drought stress in wheat seedlings whereas relative water content decreases. Similarly, some soluble carbohydrates (Kerepesi & Galiba, 2000) and proteins (Noorka *et al*., 2009) also increase in drought tolerant varieties of wheat.

The main objective of this study was to characterize three different sets of wheat genotypes for drought tolerance under control and stress conditions by analyzing their morphological and biochemical responses.

Materials and Methods

The research material is comprised of fifty-one wheat genotypes classified as D-genome synthetic hexaploid derivatives (n=10), local cultivars (n=12) and land races collected from various locations of Pakistan (n=29). The passport information of land races and pedigree of cultivars and synthetic derivatives are given in Table 1.

Analysis under *In vivo* conditions: All genotypes were planted in tunnels where the irrigation was provided twice; one week before sowing and 80 days after sowing. Randomized block design (RBD) with three replications was followed. Morphological parameters including days to heading (DH), days to physiological maturity (DM), plant height (PH), spike length (SL), number of grains per spike (GPS) and thousand grain weight (TGW) were noted under control and drought conditions.

Analysis under *In vitro* conditions: Seeds of all wheat genotypes were sown in Jiffy-7 peat pots with 20 seedlings per genotype. After seven days, the seedlings were transferred from Jiffy pots to hydroponics containing Hoagland solution. From these, 10 seedlings were used as control and 10 were subjected to drought stress by replacing the Hoagland solution with PEG-6000 (30%). The sampling was done from both drought stressed and controlled seedlings in test tubes containing particular solution for every test.

Table 1. Pedigree list of different wheat genotypes.

S. No.	Name/Pedigree	S. No.	Name/Pedigree
	Synthetic derivatives	LR16	8A (Selection)
SD1	SH DR No. 45×Seher	LR17	D-9 (Barani selection)
SD2	S.RIC-62×NR-26	LR18	C-217 (C-516×C-591)
SD3	DR.MP.1-95×NN(L)R_1-4	LR19	C-288 (Hard federation×9D)
SD4	L.SEQ.15×N(N)17R_1	LR20	C-245
SD5	S.RIC-10×NN(L)R_2-48	LR21	C-247
SD6	DR.MP.2-26×NNR_1-2	LR22	C-248 (LR 26, 14A)
SD7	S.RIC-75×Wafaq	LR23	C-250 (Hard federation×9D) (LR30, 14A)
SD8	S.RIC-51×Pastor-68	LR24	C-256 (LR 10, 23, 30)
SD9	$F_1$460 (Seq.3×Seq.4-36)×Wafaq	LR25	C-258
SD10	$F_1$484 (Seq.4-78×IBWSH152)×NN(L) R_1-8	LR26	C-269 (LR 2a, 18)
	Land races	LR27	C-271 (C-220×IP165)
LR1	T1 (*T. durum*)	LR28	C-288
LR2	T2 (*T. durum*)	LR29	C-518 (T9×8A)
LR3	T3 (*T. durum*)		**Local cultivars**
LR4	T5 (*T. spherococcum*)	V1	Inqilab-91
LR5	T7 (*T. spherococcum*)	V2	Baviacora
LR6	T8 (*T. aestivum*)	V3	Opata M85
LR7	T9 (*T. aestivum*)	V4	Suleman- 96
LR8	T12 (*T. aestivum*)	V5	Sitta
LR9	T14 (*T. aestivum*)	V6	Weebill
LR10	T15 (*T. aestivum*)	V7	Nesser
LR11	T16 (*T. aestivum*)	V8	Dharwar
LR12	T17 (*T. aestivum*)	V9	Zarghoon
LR13	T18 (*T. aestivum*)	V10	Chakwal-86
LR14	T20 (*T. aestivum*)	V11	Margalla-99
LR15	T24 (*T. aestivum*)	V12	Marwat

Physiological parameters including proline, chlorophyll, protein, sugar and SOD contents were analyzed. Proline present in wheat leaves was determined by the method of Bates *et al*. (1973), total chlorophyll content by the method of Arnon (1949), protein present in wheat leaves by the method of Lowry *et al*. (1951) using BSA (Bovine Serum Albumin) as standard, soluble sugar of fresh leaves by the method of Dubois (1951), and superoxide dismutase (SOD) content was determined by the method of Giannopolitis & Ries (1977).

Statistical analysis: For statistical analysis, all data were subjected to ANOVA using MINITAB software and the treatment means were compared by LSD test at a probability level of 0.05 and 0.01 using COSTAT software. Genotypes were compared by critical difference (CD) at 5% and 1% probability. Pearson co-efficient of correlations was performed using aforementioned software by setting probability at 5% and 1%. Significant differences between both treatments were further validated with pairwise t-test at 0.01% probability.

Results

According to the results, genotypes, treatments and their interactions differed significantly ($p=0.05$) from each other for all the traits except SOD where interaction of varieties and treatments was found non-significant (Table 2). The statistical significance indicated that the variability exists among genotypes and between both treatments. Furthermore, each genotypic response was validated to be significantly different from mean at CD 0.05 and 0.01 levels to confirm the variability among genotypes under both treatments (Tables 1 and 2). It was observed that under water stress condition, there was a significant reduction in all the yield components whereas all biochemical components except chlorophyll increased significantly to help the plant in tolerating the stress.

The association between control and drought condition for all traits was assessed by t-test. It was revealed that all morphological traits have significant negative association between both treatments and all the physiological traits have positive association except for chlorophyll content where the control and drought condition showed negative association (Table 2).

The comparative assessment of three genotypic groups is given in Table 3. The synthetic derivatives showed the minimum reduction in morphological parameters followed by local cultivars and land races, respectively. The synthetic derivatives triggered the biochemical constituents more efficiently as compared to local cultivars and land races under water stress condition induced by PEG, although a slight lower range of physiological traits was observed in them.

Table 2. Analysis of variance (ANOVA) for morphological and physiological traits under control and drought conditions.

Morphological traits	**DH**		**DM**		**PH (cm)**		**SL (cm)**		**GPS**		**TGW (g)**	
	Control	**Stress**	**Control**	**Stress**	**Control**	**Stress**	**Control**	**Stress**	**Control**	**Stress**	**Control**	**Stress**
Mean	119.96	113.45	157.6	142.39	88.88	71.6	10.56	9.07	55.65	32.74	35.47	27.36
ANOVA												
Genotypes	**	**	**	**	**	**	**	**	**	**	**	**
Treatments	**	**	**	**	**	**	**	**	**	**	**	**
G x T	**		**		**		**		**		**	
CD (0.05)	2.50	1.77	2.72	1.79	3.06	2.63	1.19	1.12	2.84	3.32	2.32	1.99
CD (0.01)	3.69	2.62	4.01	2.64	4.52	3.88	1.75	1.66	4.19	4.90	3.43	2.95
t-test	-8.13***		-11.13***		-12.21***		-8.92***		-12.56***		-9.13***	
Physiological traits	**Proline (µg/g)**		**Chlorophyll (mg/g)**		**Protein (µg/g)**		**Sugar (µg/g)**		**SOD (units/g)**			
	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress		
Mean	453.6	1431.1	0.94	0.67	1107.31	1260	554.74	687.6	25.08	29.13		
ANOVA												
Genotypes	**	**	**	**	**	**	**	**	**	**		
Treatments	**	**	**	**	*	*	**	**	*	*		
G x T	**		**		**		**		**ns**			
CD (0.05)	17.67	25.07	0.53	0.49	14.16	14.04	10.81	12.32	2.39	2.50		
CD (0.01)	26.10	37.03	0.79	0.73	20.91	20.73	15.96	18.20	3.52	3.69		
t-test	10.13***		-8.03***		5.04***		8.14***		8.92***			

*, **, *** Significant difference at p> 0.05, 0.01 and 0.001 levels, respectively.

DH, Days to heading; DM, Days to maturity; PH, Plant height; SL, Spike length; GPS, Grains per spike; TGW

Table 3. Mean and range values of three sets of genotypes under control and moisture stress conditions in field and laboratory.

Parameters		Synthetic derivatives		Local cultivars		Landraces	
		Control	Stress	Control	Stress	Control	Stress
DH	Mean	111.4	110	124.75	114.08	120.93	114.37
	Range	103-120	103-115	119-129	109-119	112-114	107-122
DM	Mean	142.6	145.5	163.02	142.66	160.51	141.19
	Range	137-152	139-155	153-170	132-149	154-171	135-150
PH (cm)	Mean	87.36	80.17	78.82	62.87	93.55	72.25
	Range	78.16-99.52	72.69-87.94	65.25-100.01	51.36-73.00	70.25-110.26	59.20-90.25
SL	Mean	11.47	9.62	11.72	10.11	9.76	8.44
	Range	9.89-13.89	7.80-12.44	8.78-14.00	7.40-13.60	6.99-12.52	5-12.10
GPS	Mean	54.10	42.37	58.47	38.52	55.01	27.02
	Range	39-60	27-68	38-77	15-55	28-74	8.0-51
TGW	Mean	39.93	29.93	37.94	27.76	32.91	26.30
	Range	29.57-52.15	22.37-32.06	29.99-48.10	22.35-35.76	24.89-42.36	17.55-38.63
Proline	Mean	258.57	2132.12	577.44	1303.2	469.6	1242.2
	Range	39.30-913.30	1171.1-4501.1	39.30-1555.70	244.70-3268.80	35.0-1485.80	332.10-3426.10
Chlorophyll	Mean	0.59	0.36	1.03	0.78	1.01	0.73
	Range	0.15-1.32	0.10-1.08	0.33-1.68	0.42-1.39	0.21-1.77	0.24-1.52
Protein	Mean	1063.31	1237.51	1254.99	1317.24	1061.37	1243.98
	Range	879.79-1356.18	859.74-1522.19	902.83-1405.10	364.78-1579.13	91.19-1405.10	282.70-2106.60
Sugar	Mean	683.96	913.26	593.54	682.82	494.12	611.78
	Range	543.07-1152.95	474.11-1367.87	307.28-836.69	434.02-943.98	253.46-817.15	275.94-1226.43
SOD	Mean	19.84	23.72	27.83	30.1	25.74	30.58
	Range	9.77-30.38	9.00-35.58	15.66-57.33	15.64-53.22	12.41-46.27	15.58-51.82

Table 4. Correlation between *In vivo* and *In vitro* parameters under control and drought stress conditions in field and laboratory.

Variables		1	2	3	4	5	6	7	8	9	10	11
DH (1)	Control	1	0.780**	-0.366**	-0.168	-0.101	-0.155	0.252	0.11	0.256	-0.360**	0.447**
	Stress	1	0.248	-0.357*	0.063	-0.165	0.012	-0.340*	0.088	0.107	-0.262	0.172
DM (2)	Control		1	-0.109	-0.224	0.075	-0.243	0.316*	0.355*	0.195	-0.471**	0.458**
	Stress		1	0.109	0.149	0.415**	0.335*	0.229	-0.305*	-0.172	0.323*	-0.131
PH (3)	Control			1	-0.002	0.447**	-0.233	-0.056	0.12	-0.331*	-0.177*	0.064
	Stress			1	0.159	0.126	0.216	0.386**	-0.109	-0.277*	0.182	-0.045
SL (4)	Control				1	0.342*	0.300*	-0.028	-0.063	0.153	0.291*	-0.075
	Stress				1	0.319	0.16	0.256	0.201	0.094	0.265	-0.014
GPS (5)	Control					1	-0.254	0.284*	0.102	-0.004	0.121	0.208
	Stress					1	0.023	0.336*	-0.164	-0.02	0.449**	-0.17
TGW (6)	Control						1	-0.135	-0.14	0.15	0.266	-0.096
	Stress						1	-0.014	-0.165	-0.114	0.254	0.089
Proline (7)	Control							1	0.410**	0.509**	-0.320*	0.577**
	Stress							1	-0.043	0.172	0.266	0.216
Chlorophyll (8)	Control								1	0.242	-0.283*	0.325*
	Stress								1	0.051	-0.463*	0.325*
Protein (9)	Control									1	-0.025	0.352*
	Stress									1	-0.047	0.195
Sugar (10)	Control										1	-0.407**
	Stress										1	-0.336*
SOD (11)	Control											1
	Stress											1

* Values are different from 0 with a significance level alpha=0.05

** Values are different from 0 with a significance level alpha=0.01

Phenotypic correlation revealed consistent relationship among traits under both treatments; however some important exceptions were found where the relationship between two traits was inversed under stress conditions (Table 4). Days to maturity and chlorophyll content showed a positive correlation (0.355**) under control condition but negative correlation (-0.305**) under stress condition. Similarly, there existed a positive correlation between days to maturity and SOD (0.458** at control; -0.131 at stress), proline and chlorophyll content (0.41** at control; -0.043 at stress) whereas negative correlation was observed between sugar and days to maturity (-0.471** at control; 0.323** at stress), sugar and plant height (-0.177* at control; 0.182 at stress) and, sugar and proline (-0.32** at control; 0.266 at stress). This indicates that these traits have considerable impact in the metabolic regulation under drought stress conditions and should be focused to dissect their independent effect on wheat adaptation to moisture stress.

Discussion

The moisture stress condition has a strong effect on all the morphological and physiological characteristics of plants. All wheat genotypes of three different sets showed diversity in their response, when compared with each other, under both control and drought environments. The decrease in days to heading due to water stress is considered effective in various bread and durum wheat varieties (Moayedi *et al*., 2010) but this does not guarantee higher yield (Ooro *et al*., 2009). We observed late maturity coupled with higher yield in case of synthetic derivative (F4-2011). Similarly, plant height, spike length, number of grains per spike and thousand grain weight were significantly decreased and showed variation under water stress conditions in various wheat genotypes (Mirbahar *et al*., 2009). Surprisingly, all synthetic derivatives had the lowest decrease in grains per spike as compared to landraces and wheat cultivars. In case of TGW, the lowest decrease was observed in landraces when compared with other genotypes. Different studies have shown that synthetics derivatives are 23% higher yielding when compared with the local cultivars (Trethowan *et al*., 2000) and this higher yield in synthetic derivatives is associated with rapid translocation of photosynthetic carbohydrates to the grains after heading time (Inagaki *et al*., 2007).

Biochemical components play a significant role in maintaining metabolic pathways during moisture stress conditions. Therefore the characterization of key biochemical components contributing to drought tolerance is a pre-requisite to identify candidate genetic resources for trait improvement. Different plants accumulate proline (Ozturk and Szaniawiski, 1981; Ozturk *et al*., 1986; Ashraf & Foolad, 2007), proteins (Demirevska *et al*., 2008), soluble sugars (Kameli & Losel, 1993) and oxidative enzymes (Bergmann *et al*., 1999) in response to various abiotic stresses, which protect the plant by reducing the oxidative damage caused by osmotic stress. In different tetraploid and hexaploid wheats, chlorophyll content decreases significantly under stress environment (Tas & Tas, 2007) and the same is observed in this study. Studies in durum wheat landraces revealed that SOD and chlorophyll content index increased in resistant landraces under drought stress condition as compared to the sensitive landraces (Zaefyzadeh *et al*., 2009).

The correlation analysis enabled to identify the traits which responded differentially under control and drought stress. This identified key traits, which can be manipulated simultaneously when using these genetic resources as a source for wheat genetic improvement. It revealed that spike length had significant positive correlation with grains per spike and non-significant positive correlation with thousand-grain weight. This non-significant positive correlation of GPS with TGW was also observed by other scientists in wheat genotypes under drought stress conditions (Munir *et al*., 2007). Studies have also shown that DM has negative correlation with chlorophyll content and SL has positive correlation with GPS (Kilic & Yagbasanlar, 2010). Similarly, protein content has negative correlation with TGW (Rharrabtia *et al*., 2003), which is also observed in our case. This interrelationship among different traits indicate that they are controlled by some common genes so if one trait is selected positively, it will also improve other correlated traits (Munir *et al*., 2007). The interrelationship among different traits studied here reveals that grains per spike and thousand-grain weight of wheat genotypes can be efficiently increased by obtaining maximum expression of spike length, proline and sugar content under stress condition. Similarly, days to maturity also plays a significant role in the yield of wheat genotypes under moisture stress condition.

The comparative analysis across three genotypic groups proves that synthetic derivatives are comparatively more tolerant than the other two groups. The synthetic wheats, which provide a bridge between genetic variation in the D-genome progenitor and modern hexaploid wheat, offer wheat breeders the greatest potential for yield enhancement under stress in the short to medium term. These primary synthetics are directly crossable with adapted wheat and potentially combine genetic variation that has not been previously existed in the hexaploid wheat gene pool. The use of synthetic wheats in CIMMYT in applied wheat breeding has not only improved stress adaptation (Trethowan & Mujeeb-Kazi, 2008), but has also significantly increased the genetic diversity of recently developed wheat germplasm (Warburton *et al*., 2006). The landraces, while offering considerable variation, are less likely to carry novel genes for abiotic stress tolerance as they, like modern cultivated wheat, are derived from the same crosses that gave rise to hexaploid wheat 8000 years ago. Nevertheless, the collection, characterization, and maintenance of these materials are essential, not only as a source of potentially new genetic variation, but due to the possibility of their extinction (Teklu & Hammer, 2006).

Conclusively, out of 51 genotypes studied, 37 performed optimum under drought stress conditions. These included all the synthetic derivatives except SD8; Suleman-96, Sitta, Weebill, Chakwal-86, Margalla-99 and Marwat from local cultivars and LR16, LR17, LR18, LR20, LR29, LR33, LR35 and LR39 from land races. From these three sets of genotypes, the top 13 genotypes were SD10, SD1, SD7, SD3, SD9, SD2, Suleman-96, Chakwal-86, Sitta, Margalla-99, Marwat, LR34 and LR8. All these genotypes showed higher yield and stability of biochemical constituents during moisture stress conditions and thus have better osmo-regulation mechanism to tolerate water deficit conditions under field conditions. The higher number of drought tolerant synthetic derived wheat lines is a good example of genetic resources conservation where the accessions of *Ae. tauschii* are conserved through production of amphiploids via bridge crosses and utilized in practical wheat breeding to improve drought tolerance.

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