Diversity Among Synthetic Back-Derived Wheat (*Triticum Aestivum* L.) Lines for Drought Tolerance

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Abstract

Wheat (*Triticum aestivum* L.) is predominantly growing in areas where terminal drought stress is the foremost yield limiting factor. Since genetic variability for climate resilience is weakened in modern wheat cultivars, exploring drought tolerance potential of the synthetic back-derived wheat lines (SBL) might help to widen genetic diversity for wheat breeders. The variation among 45 selected SBLs in response to drought stress was assessed by measuring physiological traits. The results showed a significant variation among genotypes under induced drought stress at reproductive stage in comparison to commercial wheat varieties as check. The variability among genotypes was verified by correlations, principal component analysis, heat map and clustering methods using Ward's method. The study selected 12 synthetic back-derived wheat lines with significantly higher grain yields than 5 local checks under drought stress. The drought tolerance ability of these selected genotypes was strongly associated with proline, leaf water contents, membrane stability and better leaf chlorophyll contents under drought stress. The identified tolerant synthetic back-derived wheat lines may prove useful for wheat breeders to evolve climate resilient wheat varieties.

Introduction

Wheat (*Triticum aestivum* L.) is an important staple food and main source of energy, contributing 20% calories, globally. The low latitude agro-ecological zone contribute about 50% of the global wheat cultivated area (Easterling and Apps 2005) and is exposed to global warming in relation to food security (Lobell et al. 2008). The recent extreme weather happenings indicate the vulnerability of low latitude cropping zones worldwide to adverse effects of climate change (IPCC 2014). The drought, a period of no or less rainfall, is the most detrimental climatic factor limiting crop growth and yield losses between 15-75% depending on a plant growth stage exposed to drought and its severity (Sohail et al. 2014). The wheat production is anticipated to decline, especially in the tropics and sub-tropics due to frequent droughts (Knox et al. 2012).

Although, the moisture stress may adversely affect any stage of the wheat growth cycle but stress during reproductive growth stages is more detrimental (Pradhan et al. 2012). A mild drought at anthesis can cause up to 30% yield losses, however, yield losses may range between 50 – 75% in case of prolonged or severe drought during reproductive stages. These yield losses are primarily due to leaf senescence (Yang et al. 2001), oxidative stress damage to photosystem II, reduced assimilation rate, less CO₂ availability, slow translocation of solutes (Asada 2006; Farooq et al. 2009) and ultimately reduced sink capacity (Liang et al. 2001).

The drought stress damage starts with gradual degradation of leaf chlorophyll contents (Sohail et al. 2014) membrane leakage, and declining water content (Hafsi et al. 2000; Chandler 2001). The disrupted leaf structure and function lead to a drop in photosynthesis (Yang et al., 2001; Gregersen and Holm 2007). Drought stress at flowering stage decrease pollen viability (Dorion et al. 1996; Cattivelli et al. 2008) which leads to spike sterility (Sohail et al. 2019) and low seed setting (Ahmadi and Baker 2001).

To enhance environmental stress tolerance in cultivated wheat cultivars, it is extremely important to assess the diversity in the stress reaction types of wheat germplasm and to identify genotypes with better levels of climatic adaptability. It is also important to identify and describe the various physiological and phenotypic traits involved in tolerance and to identify the genetic components behind tolerance mechanisms (Sohail et al. 2019). Since considerable genetic variability for environmental stress tolerance is declining in modern cultivated wheat, exploring of novel diversity is needed to overcome narrow genetic base in wheat.

There is necessity to exploit the sources of genetic diversity contained in primary synthetics and wild relatives for future gains in yield potential (Skovmand et al. 2001) under abiotic conditions

Maes et al. (2001) and Yang et al. (2002) observed genetic diversity in primary wheat synthetics for abiotic stress tolerance. The Gororo et al. (2002) and Trethowan et al. (2005) reported better grain weight of synthetic wheat genotypes grown under drought stress. There are advantages of better grain yield and biomass production synthetic derived wheat lines over their parents, which are linked with their more responsive root system to drought (Reynolds et al. 2007) reported. The Lopes and Reynolds (2011) also stated the improved range of stress adaptive traits in synthetic wheat derivatives under drought situation.

The synthetic wheat derivatives have a broader genetic base and can, therefore, provide needed basis to evolve drought tolerant commercial wheat cultivars. The objective of the study was to evaluate synthetic backcross derived wheat lines as potential genetic resources for drought tolerance and widen wheat genetic diversity for climate resilience. It was also hypothesized that certain physiological traits like proline contents, leaf water contents and membrane stability under drought stress tends to be correlated with grain yield and important yield contributing agronomic traits i.e. kernels per spike and kernel weight, therefore they can be used as selection markers in stress breeding programs.

Material And Methods

Plant material, treatments and growing conditions

The study screened 50 bread wheat genotypes comprising 45 synthetic back-derived wheat lines (SBL) and 5 commercial varieties as checks, release for rainfed ecology. All the all tested wheat germplasm was acquired from Wheat Program, Crop Sciences Institute, National Agricultural Research Centre, Islamabad Pakistan. The Synthetic backcross-derived wheat lines (SBLs) were developed by primary synthetic hexaploid wheat crossed with improved cultivars from Pakistan and CIMMYT. The wheat lines were chosen based on their diverse genetic background or pedigrees. The detail of the wheat germplasm, utilized in this study, is provided in the Table 1. The experiment was carried in randomized complete design (RCD), comprising two treatment i.e. normal and drought stressed environments, 50 genotypes and 03 replications. The trial was conducted in greenhouse conditions during 2018/19 at the

greenhouse house facility of Plant Physiology Program, Crop Sciences Institute, National Agricultural Research Centre, Islamabad. The day/night temperatures of greenhouse were maintained at 30 °C/20 °C with air humidity ranged from 40-60%.

Table 1
The detail of wheat lines used in this study

Entry No.	Line code*/name	Entry No.	Line code*/name
1	SBL 1	26	SBL 26
2	SBL 2	27	SBL 27
3	SBL 3	28	SBL 28
4	SBL 4	29	SBL 29
5	SBL 5	30	SBL 30
6	Pakistan 2013	31	SBL 31
7	SBL 7	32	SBL 32
8	SBL 8	33	SBL 33
9	SBL 9	34	SBL 34
10	SBL 10	35	SBL 35
11	SBL 11	36	SBL 36
12	Shahkar 2013	37	Ehsan 2016
13	SBL 13	38	SBL 38
14	SBL 14	39	SBL 39
15	SBL 15	40	SBL 40
16	SBL 16	41	SBL 41
17	SBL 17	42	SBL 42
18	SBL 18	43	SBL 43
19	SBL 19	44	Barani 2017
20	SBL 20	45	SBL 45
21	Faisalabad 2008	46	SBL 46
22	SBL 22	47	SBL 47
23	SBL 23	48	SBL 48
24	SBL 24	49	SBL 49
25	SBL 25	50	SBL 50
*The detailed pedigree/parentage is provided in supplementary table 1. The position of each line in this table and supplementary table information is completely corresponds to their positions in the all statistical analysis in this study.			

The drought stress during reproductive stage was induced by withholding water application and maintaining moisture contents of the pots to 35% field capacity from heading to physiological maturity. The experiment was conducted in plastic pots of 8 litre capacity filled with loam soil consisting sand (60%), silt (25%) and clay (15%). Seven seeds of each genotype were sown in each pot and after thinning at 3 leaf stage, 3 plants were maintained in each pot till maturity with all essential agronomic practices.

Data collection

The physiological data was recorded on the chlorophyll content (SPAD), relative water contents (RWC), membrane stability index (MSI) and proline contents (PC) in green tissues of flag leaves just after 15 days of drought treatment.

The leaf chlorophyll contents were recorded using a SPAD-502 device (Minolta, Japan). The instrument measures leaf transmittance in the red and near infrared spectra and consequently calculates the SPAD index. Five readings of the chlorophyll content were measured per pot on the middle of the flag leaf and then averaged out into a single SPAD value.

The mature and fully extended flag leaves were cut by scissor from five random tillers per pot and immediately placed in ice box for relative water content (RWC) determination. The leaves were cut into fragments and instantly taken fresh weight (FW). The leaves were then submerged in distilled water for 24 hours and turgid weight (TW) of saturated leaves was recorded just after 24 hours of submergence. The dry weight (DW) of the leaves was recorded after keeping samples in oven at 80°C for 24 hours. The relative water content was determined using following formula.

Relative Water Content = $FW - DW / TW - DW \times 100$ (Barrs and Weatherley 1962)

Membrane Stability Index (STI) of leaf tissues was determined by recording the electrical conductivity (EC) of leaf leakages in double distilled water at 40°C and 100°C. The randomly taken five flag leaves were washed with distilled water and were chopped into 3 cm long pieces. Two sets of well washed test tubes were made, one as control and other containing drought treated leaves, each having 20 ml of distilled water and 200 mg of leaf samples. The test tubes were wrapped with paraffin film and heated in water bath at 40°C for 30 min. (T1). The tubes were then kept at 15°C for 24 hours to allow leakage of electrolytes from leaf samples. Later on tubes were shifted to ambient temperature, shaken well and electric conductivity (C1) was measured. The other set was put in a boiling water bath at 100°C for 15 minutes and then cooled to 25°C and final conductivity (C2) was recorded (Deshmukh et al 1991).

The membrane stability index (MSI) was calculated as: $MSI = [1 - (C1 / C2)] \times 100$

The proline content was determined by using acid-ninhydrin method developed by the Bates et al (1973). Fresh leaf samples were harvested of the second top leaves from the drought treated and control pots and immediately placed in the ice box. The fresh leaf samples (0.1 g) were ground in 10 ml of 3% aqueous sulfosalicylic acid and then allowed to settle for two hours. Then 2 ml of supernatant was mixed with 2 ml glacial acetic acid and 2 ml of the acid ninhydrin reagent and whole mixture was boiled for one hour at 100°C in a water bath. Afterward, the test tubes were taken out from water bath and permitted to cool at room temperature. Finally, 4 ml of Toluene was added to the reaction mixture. Then, the absorbance for proline was read at 520 nm UV-visible spectrophotometer (UV-1800 spectrophotometer, Shimadzu Corporation, Kyoto, Japan). The proline contents were determined using the following formula:

Proline Content = $Absorbance\ of\ Sample \times K\ value \times Dilution\ factor / Weight\ of\ sample \times 100$

The pots were harvested at harvest maturity and agronomic traits including kernels per spike (KPS), thousand kernel weight (TKW) and grain yield (GY) of all wheat genotypes were recorded according to procedure described by the Pask et al (2012)

Statistical analyses

The significance of differences between treatments means were calculated using analysis of variance (ANOVA) technique, appropriate for two factors, in complete randomized design (CRD) using statistical software Statistica 7 (Stat Soft, USA). The statistical software also helped to compute summary statistic, the significance of differences between treatments means (Tukey's test) and Pearson's correlation analysis to develop the correlation matrix. The association among performance of genotypes and phenotypic traits was further find out using principal component analysis (PCA) using XLSTAT software (Addinsoft 2014). The PCA biplot was drawn between first two principal components (PC1 and PC2) to visualize the results. The heatmap with hierarchical clustering (ward's method) of wheat genotypes based on the mean values of physio-agronomic traits was performed using R ver. 3.4.1 software package (R Core Team 2018).

Results

ANOVA and descriptive statistics

The analysis of variance results indicated significant ($P < 0.05$) effects of the environment (drought stress treatment), genotype and environment x genotype interactions for the most of the studied physio-agronomic traits (Table 2). The drought stress treatment (environment) significantly affected SPAD (leaf chlorophyll value), RLWC (relative leaf water contents), MSI (membrane stability index), PC (proline content), KPS (kernels per spike), TKW (thousand kernel weight) and GY (grain yield). The ANOVA results also showed that there is significant variability among the studied genotypes with regard to physio-agronomic traits under both normal and drought condition. Similarly, the effect of the environment x genotype interactions was also significant for SPAD, RLWC, PC, TKW and GY, however, MSI and KPS were not significantly influenced (Table 2).

Table 2
Mean square and Analysis of variance (ANOVA) for the physio-agronomic traits of the 50 synthetic back-derived wheat lines evaluated for drought tolerance at reproductive stage.

Source of variation	DF	Physio-agronomic traits						
		SPAD	RLWC	MSI	PC	KPS	TKW	GY
Environment	1	13101**	24604.1**	41550.9**	2927.44**	16995.2**	9185.33**	140660**
Genotype	49	60.0**	124.7**	12.4 ^{ns}	3.49**	11.6 ^{ns}	10.81**	362**
Environment x Genotype	49	64.2**	125.4**	20.4 ^{ns}	3.76**	13.4 ^{ns}	10.37**	334**
Error	198	3.7	10.8	18.4	0.64	9.8	1.75	42
*, ** indicates significance at $P < 0.05$, $P < 0.01$ respectively and ns = non-significant								
Abbreviations: SPAD, leaf chlorophyll value; RLWC, relative leaf water contents; MSI, membrane stability index; PC, proline content; KPS, kernels per spike; TKW, thousand kernel weight; GY, grain yield;								

The basic statistical summary of means with standard deviation, range, relative traits change and coefficient of genetic variance for the physio-agronomic traits of the 50 synthetic back-derived wheat lines evaluated for drought tolerance at reproductive stage are provided in the Table 3.

Table 3

Mean values, range, relative traits change and coefficient of genetic variance for the physiological and agronomic traits of the 50 synthetic back-derived wheat lines evaluated for drought tolerance at reproductive stage.

Physio-agronomic traits	Normal		Drought stress		RTC	CGV
	Mean ± SD	Range	Mean ± SD	Range		
SPAD value	50.4 ± 2.1	46.8 - 53.2	37.2 ± 6.4	29.5 - 50.1	0.26	23.3
RLWC (%)	81.6 ± 2.3	76.5 - 84.7	63.5 ± 9.5	44.3 - 79.3	0.22	30.6
MSI (%)	80.5 ± 2.2	76.6 - 85.0	58.1 ± 5.5	44.3 - 65.3	0.28	22.8
PC (μmol per g FW)	2.30 ± 0.6	0.5 - 3.9	8.5 ± 1.6	4.4 - 12.4	-2.70	9.30
KPS (no.)	48.6 ± 3.9	43.0 - 57.0	33.5 ± 2.3	30.0 - 40.0	0.31	18.2
TKW (g)	34.7 ± 1.2	33.0 - 37.0	23.6 ± 2.8	19.0 -30.0	0.32	22.7
GY (g/pot)	50.0 ± 1.9	43.5 - 53.4	36.6 ± 1.4	28.6 - 45.0	0.27	23.5
Abbreviations: SPAD, leaf chlorophyll value; RLWC, relative leaf water contents; MSI, membrane stability index; PC, proline content; KPS, kernels per spike; TKW, thousand kernel weight; GY, grain yield; RTC, Relative traits change; CGV, coefficient of genetic variance.						

The relative traits change (RTC) values of physio-agronomic traits were calculated as relative difference of their mean values under control and drought conditions. The RTC of measured traits, were recorded in the pattern, i.e., GY (0.37) > TKW (0.32) > RLWC (0.28) > MSI (0.26) > SPAD (0.23) > KPS (0.21). Nevertheless, the PC showed inverse RTC value (-2.70) as it is increased under drought stress environment in comparison to other measured traits. The coefficient of genetic variation (CGV) of physio-agronomic traits of the 50 synthetic back-derived wheat lines, tested under normal and drought stressed environments, was ranged from 9.30 to 30.6% (Table 3). The genetic variation of some traits i.e. MSI, RLWC, TKW and GY was >20%, while genetic variation of SPAD and KPS was ranged between 10% and 20%. The genetic variation coefficient for PC was <10% (Table 3). These results indicated high genetic variation within the studied synthetic back-derived wheat lines under both normal and drought stress condition.

The drought stress had higher reducing effects on pooled means of grain yields of all 50 wheat lines. The grain yields of genotypes are ranged from 43.5 to 53.4 under normal conditions with a mean value of 50.0 (Table 2). The fifteen 15 SBL showed higher while 20 lines showed lower GY than check varieties (cultivated commercial varieties). However, under drought stressed condition, The GY of the genotypes is ranged from 28.6 to 45.3 with a mean value of 36.6. The 35 SBL indicated higher and 10 SBL showed lower yield as compared to commercial checks (Fig. 1). The results showed considerable potential of synthetic back-derived wheat lines for genetic improvement and could be useful sources of drought tolerance.

Correlations of physio-agronomic traits

Correlation coefficients (r) describing the level of correlations among observed physiological and agronomic parameters are summarized in Table 4. The grain yield, under normal growing conditions, showed a positive and significant correlation ($r = 0.35^*$) with TKW, while correlation with physiological parameters like SPAD, RWC, MSI was weak positive and non-significant ($P > 0.05$). The grain yield, under drought stress condition, showed a strong positive ($r > 0.7$) and significant ($P < 0.05$) correlations with TKW, KPS, SPAD, RLWC, MSI and Proline.

Table 4

Correlation coefficients (Pearson's) among agronomic and physiological traits of 50 synthetic backcross derived (SBL) wheat lines evaluated under drought stressed and normal condition.

		Normal						
	Traits	GY	KPS	TKW	SPAD	PC	MSI	RLWC
Drought	GY	1	0.045	0.351*	0.133	-0.217	0.090	0.065
	KPS	0.872**	1	0.061	-0.042	-0.214	0.100	-0.183
	TKW	0.882**	0.884**	1	-0.340*	0.074	-0.035	0.040
	SPAD	0.908**	0.890**	0.889**	1	-0.076	-0.147	-0.129
	PC	0.785**	0.777**	0.870**	0.843**	1	-0.130	0.020
	MSI	0.883**	0.849**	0.839**	0.877**	0.790**	1	0.263
	RWC	0.864**	0.840**	0.849**	0.842**	0.821**	0.868**	1

*, ** indicates significance at $P < 0.05$, $P < 0.01$ respectively

Abbreviations: SPAD, leaf chlorophyll value; RLWC, relative leaf water contents; MSI, membrane stability index; PC, proline content; KPS, kernels per spike; TKW, thousand kernel weight; GY, grain yield;

TKW did not show any strong correlation with measured physiological traits under normal conditions, however, it showed a strong positive correlation ($r > 0.7^{**}$) with SPAD, MSI, RLWC and PC under drought stressed environment.

Similarly, KPS did not show any significant correlation with recorded agronomic and physiological parameters under normal conditions, but showed strong positive correlations ($r > 0.7^{**}$) with all physio-agronomic traits under drought stress.

Principal Component Analysis (Pca)

The fraction of the overall variance expounded by different principal components and their associations with variable traits is shown in the rotated component matrix (Table 5). Two principal components were contributing 91.74% of the total variation noticed under drought stress treatment. Nevertheless, the first principal component was most important with a cumulative contribution of 88.06% to the total variation. All physio-agronomic variables like SPAD, MSI, RLWC, PC, KPS, TKW and GY had high positive loading into the first principle component. Under normal growing environment, three principal components were important, recording for 73.66% of the total variation. SPAD, MSI and TKW had high positive loading into the 1st, while, KPS and GY had high positive loading into the 2nd principal component. However, high positive loading of the Proline and RLWC was noticed into the 3rd principal component.

Table 5
Rotated component matrix of agronomic and physiological traits of 50 synthetic backcross derived (SBL) wheat lines evaluated under drought stressed and normal conditions.

	Drought			Normal		
Traits	PC-1	PC-2	PC-3	PC-1	PC-2	PC-3
SPAD	0.955	-0.024	-0.158	0.519	-0.143	0.166
PC	0.898	0.421	0.038	0.094	-0.554	0.229
MSI	0.934	-0.159	0.168	0.445	0.083	0.540
RWC	0.932	-0.024	0.292	-0.426	-0.396	-0.214
KPS	0.947	-0.123	-0.134	-0.249	0.599	0.260
TKW	0.954	0.098	-0.134	0.432	-0.103	-0.512
GY	0.948	-0.170	-0.061	0.306	0.373	-0.503
Eigenvalue	6.165	0.258	0.180	1.684	1.363	1.205
Total variance (%)	88.065	3.682	2.565	24.064	19.475	17.217
Cumulative variance (%)	88.065	91.747	94.312	24.064	43.539	60.756
Abbreviations: SPAD, leaf chlorophyll value; RLWC, relative leaf water contents; MSI, membrane stability index; PC, proline content; PC-1, principal component 1; PC-2, principal component 2; PC-3, principal component 3; KPS, kernels per spike; TKW, thousand kernel weight; GY, grain yield						

The biplot analysis (Fig. 2) illustrates the associations between the different variables and genotypes with respective principal components for the normal (Fig. 2; a) and drought stress conditions (Fig. 2; b). The acute angles (<90°) between dimension vectors in the similar direction showed positive correlation of the variable traits in terms of describing genotypes. The genotypes outrivaling in a specific trait were plotted nearer to the vector length. The angles between dimension vectors of GY and other yield related traits like KPS, TKW, RLWC, MSI, PC and SPAD showed positive correlation (<90°) under drought stress (Fig. 2; b). However, under normal conditions, GY was only positively correlated with TKW, KPS, SPAD and MSI (Fig. 2; a). Moreover, PCA biplot analysis helped grouping tested wheat lines according to their similarities in yield contributing traits correspondences under drought stress. Most of the better performing genotypes under drought stress environment (tolerant) were concentrated in the positive side of the first principal component (Fig. 2; b). These genotypes excelling in overall productivity over check varieties was contributed mostly by high TKW, RLWC and optimum values for other physio-agronomic traits as well, nevertheless, the genotypes were more scattered on the both sides of the two principal components under normal condition (Fig. 2; a).

Cluster analysis and heat map

The heatmap and cluster analysis of the mean values of physio-agronomic traits of the wheat genotypes under stress and normal conditions was performed. The heatmap was created based on the standardized Z-score values and the hierarchical clustering was performed using ward's method. The similar z-score colour and the distance between clusters show the similarity of the genotypes (Fig. 3). The heatmap and ward's hierarchical clustering classified SBLs into three distinct clusters, i.e., cluster 1 with negative Z-score (dark blue colour) was comprised of 21 SBLs which showed less mean yield attributes and recognized as drought sensitive lines i.e. SBLs 25, 23, 19, 28, 34, 39, 41, 46, 47, 08, 31, 43, 07, 42, 14, 45, 48, 33, 49, 18 and 20. The group 2, comprised of 17 SBLs with strong positive Z-score (dark yellow colour) for yield contributing traits is considered as drought tolerant group. It include SBLs 21, 05, 02, 37, 09, 32, 40, 06, 16, 12, 17, 44, 50, 04, 30, 22 and 26. The 3rd group (13 SB lines) showed weak positive Z-score (light yellow colour) and accepted as moderate drought tolerant group (Fig. 3).

Discussion

Overall climate change has an adverse impact on crop production and the global food security and there has been professed a certain decrease in yield of crops for last two decades (McKersie and Leshem 1994; Ashraf 2010). Development of climate resilient wheat cultivars is the prime objective of wheat breeders and they are persistently improving germplasm of crop plants for drought tolerance by selection methods and genetic modification (Ehlers and Goss 2003; Fleury et al. 2010)

The available wheat germplasm have narrow genetic diversity for abiotic stress tolerance (Feuillet et al. 2008), exploring synthetic backcross derived wheat lines (SBL) for drought tolerance potential is a substantial approach to widen wheat genetic diversity for advanced wheat breeding programs. The germplasm panel (synthetic backcross derived wheat lines) used in this study showed highly significant variation for all recorded physiological and agronomic traits indicate that it could be a rich source of genetic diversity for breeding objective of drought tolerance. The observed significant effects of the environment, genotype and environment x genotype interactions for the most of the phenotypic traits were expected. Better growth and grain yield (GY) of certain synthetic backcross derived wheat lines (SBL) than local checks was linked with their superior scores of some physiological and agronomic yield contributing traits like relative leaf water contents (RLWC), leaf chlorophyll (SPAD) and thousand kernel weight (TKW) under drought stress. These findings endorse the use of SBL to evolve drought tolerant wheat cultivars. Similar results were also reported by Lopes and Reynolds (2011) for a set of elite lines derived from synthetic hexaploid wheat under water-stressed field conditions.

The positive and significant correlations ($r > 0.5$) of GY with RLWC, SPAD, MSI, proline contents (PC), KPS and TKW under water stress conditions indicate the direct impact of these yield contributing components to grain yield. The results showed strong contribution of these physio-agronomic traits towards grain yield under moisture stressed conditions, meanwhile, all yield components equally contributed to grain yield under optimum conditions. Dodig et al (2012) and Sareen et al (2014) also inferred related findings after screening wheat accessions and land races for drought tolerance potential. It is also reported that the tolerant genotypes managed to mitigate drought stress damage by maintaining better leaf water contents, leaf chlorophyll, number of grains per spike and grain weight than others (Slafer et al, 2014; Sohail et al. 2019). The results implying that selection for these physio-agronomic traits could significantly improve the yields and these parameters should be used as important target traits while screening genotypes for drought tolerance.

The Principal Component Analysis (PCA) also indicated that the measured physio-agronomic traits are strongly linked with grain yield. The analysis highlights the significance of screening genotypes based on physio-agronomic traits which could result in simultaneous selection for instincts physiological mechanisms tallying up to productivity under drought stress. The higher positive loading of physiological traits i.e. SPAD, RLWC, Proline and MSI into the first principal component under drought stress indicate that they are much influential physiological traits and have direct impact on each other. They might simultaneously be considered as selection criteria for drought tolerance potential of genotypes.

The synthetic backcross derived wheat lines evaluated in this study show a relatively wide range of drought stress tolerance linked to variability in yield contributing physiological and agronomic traits. It could be inferred from the results that wheat lines with better physiological traits have efficient photo-assimilate production and biomass accumulation under drought stress and ultimately will have higher grain yields. The results of this study are consistent with some erstwhile reported results (Golabadi et al. 2006; Mohammadi et al. 2010; Sohail et al. 2014) conducted on wheat.

The decreased water content in plant tissues, under drought conditions, mars various bio-physiological processes, especially the adverse changes in photosystem and photosynthesis. The water limited conditions in plant cell primarily results a stomata closure, as plant tries to reduce water loss from plant tissues, resulting in a decline in the availability of CO₂ and consequently in a reduction of photosynthetic efficiency (Lawlor and Cornic 2002). The drought stress can also induce changes in other bio-physiological mechanisms like chlorophyll synthesis and changes in structure and function of chloroplasts, disorder in accumulation of assimilates (Medrano et al. 2002; Flexas and Medrano 2002)

In our study, under normal growing conditions, the SBL maintain higher leaf water status and chlorophyll in order to have photosynthesis process at an optimal level. But under drought conditions, genotypes reduce the leaf water and chlorophyll contents up to varying levels with significant variation. At the same time, proline contents of the leaf tissues enhanced which might be an indicator of the osmotic adjustment to maintain stability of the PSII complex and ultimately photosynthesis during drought.

Using PCA and heatmap with hierarchical clustering (Ward's methods) it appears to be possible to rank tested genotypes into tolerant, moderately tolerant and susceptible to drought stress on the basis of grain yield and associated yield contributing phenotypic traits in both drought stressed and normal environment. The outputs of PCA and univariate statistics allowed to classify the groups of similarity and indicated that drought tolerance potential of some similar genotypes have common intrinsic genotypic traits. In drought tolerant SBLs, grain yield reduction was significantly lower as compared to others and genotypes also maintained relatively higher leaf chlorophyll and water contents in their leaf tissues.

The ability of the genotypes to protect the structure and function of cytoplasmatic membranes under stress is one of the most essential physiological traits. The membrane stability index i.e. conductometric measurement of membrane injury or ion leakage was applied as a screening trial for the estimation of tolerance to drought stress (Palta 1990). The significantly higher MSI values of tolerant SBLs under drought stress indicate that tolerant genotypes possess intrinsic physiological mechanisms to protect membrane function and structure (Szechyńska-Hebda et al. 2016). Our results and other similar research findings indicate that membrane stability index measured of electrolyte leakage from fresh plant tissues is an effective tool to assess level of sensitivity of the genotypes to drought stress. These results confirm that drought stress tolerant genotypes can develop more effective mechanisms to protect cell membranes than sensitive genotypes through osmotic adjustment.

The application of different multivariate statistical analysis of results in this study, i.e. principal component, heatmap and hierarchical clustering, ranked or classified tested SBLs more or less similarly as tolerant, moderate or sensitive under stressed conditions. Therefore, our results endorse the inference of other researchers (Talebi et al. 2009; Zhang et al. 2011) about the effective utilization of these approaches in the grouping and ranking of genotypes for tolerance and sensitivity to abiotic stresses.

In conclusion, this study explored the potential of the synthetic backcross derived wheat lines (SBLs) for drought tolerance and found the existence of a wide range of genotypic variability among tested genotypes. The findings contribute to identify drought tolerant SBLs (21, 05, 02, 37, 09, 32, 40, 06, 16, 12, 17, 44, 50, 04, 30, 22 and 26) which might have intrinsic bio-physiological mechanisms to tolerate drought stress. The physiological traits determination like Leaf chlorophyll, leaf water contents, membrane stability index and proline contents served as useful tools to screen wheat germplasm for drought stress; a

physiological approach to crop breeding for drought stress tolerance. The PCA, heatmap and associated univariate statistical analysis were convenient to understand the complex multidimensional responses of the genotypes and classification based on tolerance level under stress. The promising lines have been marked based on better yield and yield contributing traits under drought stress and recommended for use in wheat breeding for drought tolerance. Our results strengthened the confirmation for the importance of synthetic back-derived wheat lines as valuable genetic resource for widening the gene pool of bread wheat and to be incorporated in the wheat breeding program to enhance climate resilience adaptability of cultivated wheat.

Declarations

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Author Contributions

M Sohail perceived, designed the experiment, collected data, analysed the results and wrote this article. M Qamar assisted in data collection, laboratory assays and facilitated in conducting the research. I Hussain served as a research supervisor aided in obtaining the funding and final review of the manuscript version to be submitted.

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Data availability

The data generated and analyzed during the current study are available from the corresponding author on reasonable request

Conflict of interest

The authors have no conflicts of interest or competing interests.

Consent for publication

All authors consent to publish.

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Figures

Figure 1

Frequency distribution of grain yield of 45 SBL against 5 check commercial varieties under normal and drought stress condition.

Figure 2

Principal component analysis Biplot. (a) PCA biplot for traits and genotypes studied under normal and (b) PCA biplot for traits and genotypes studied under drought stress treatment. Abbreviations: SPAD, leaf chlorophyll value; RLWC, relative leaf water contents; MSI, membrane stability index; PC, proline content; KPS, kernels per spike; TKW, thousand kernel weight; GY, grain yield

Figure 3

Heatmap and hierarchical clustering (ward's method) of the mean values of physio-agronomic traits of the wheat genotypes under normal and drought stress condition. The similar z-score color and the distance between clusters show the similarity of the genotypes. Abbreviations: SPAD, leaf chlorophyll value; RLWC, relative leaf water contents; MSI, membrane stability index; PC, proline content; KPS, kernels per spike; TKW, thousand kernel weight; GY, grain yield