



Diversity among synthetic backcross-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 drought tolerance is weakened in modern wheat cultivars, exploring the drought tolerance potential of the synthetic backcross-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 physio-agronomic traits under induced drought stress at the reproductive stage in comparison to 5 known drought tolerant commercial wheat varieties as check. The variability among genotypes was verified by analysis of variance, principal component analysis, a heat map, and clustering methods using Ward's method. The study selected 12 SBLs 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 SBLs may prove useful wheat lines for wheat breeders to evolve drought-tolerant wheat varieties.

Keywords Bread wheat · Genetic diversity · Physiology · Drought stress · Drought tolerance

Introduction

Wheat (*Triticum aestivum* L.) is an important staple food and main source of energy, contributing 20% of calories, globally. The low latitude agro-ecological zone contributes 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, whose extent and magnitude impacts are larger than estimated in previous assessments, indicate the vulnerability of low latitude cropping zones worldwide to severe adverse effects of climate change (IPCC 2022). The drought, a period of no or less rainfall, is the most detrimental climatic factor limiting wheat growth and it reduces yield between 15 and 75% depending on a plant growth stage exposed to drought and its severity (Sohail et al. 2014). These yield losses are primarily due to oxidative stress damage to photosystem II, reduced assimilation rate, less CO₂ availability, slow translocation of salutes (Asada 2006; Farooq et al. 2009), and ultimately reduced sink capacity (Liang et al., 2010; Yang et al. 2001). Wheat production is anticipated to decline, especially in the tropics and sub-tropics due to frequent droughts (Knox et al. 2012).

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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 the flowering stage decreases 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 drought tolerance ability in cultivated wheat, one first important step is to explore the genetic diversity of available wheat germplasm for drought tolerance. 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 there is a need to enhance and explore available genetic variability for drought stress tolerance in wheat under changing climate scenarios. There is a 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 drought condition. Maes et al. (2001) and Yang et al. (2002) observed genetic diversity in primary wheat synthetics for abiotic stress tolerance. 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 of synthetic derived wheat lines compared to their parents, which are linked with their more responsive root system to drought (Reynolds et al. 2007). Lopes and Reynolds (2011) also stated the improved range of stress adaptive traits in synthetic wheat derivatives under drought situations.

The synthetic wheat derivatives have a broader genetic base and can, therefore, provide the needed basis to develop 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 drought tolerance. It was also hypothesized that certain physiological traits like proline contents, leaf water contents, and membrane stability under drought stress tend 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.

Materials and methods

Plant material, treatments, and growing conditions

The study screened 45 synthetic backcross-derived wheat lines along with 5 drought tolerant commercial wheat varieties, release for rainfed ecology, as checks (Table 1). These checks were Pakistan-2013, Shahkar-2013, Faisalabad-2008, Ehsan-2016 and Barani-2017. All the tested wheat germplasm was acquired from Wheat Program, Crop Sciences Institute, National Agricultural Research Centre, Islamabad Pakistan. The synthetic backcross-derived wheat lines 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 Table 1. A pot experiment was carried out in randomized complete design (RCD), comprising two treatments i.e., normal and drought-stressed environments, and 50 genotypes. The five wheat seeds, surface sterilized with 5% sodium hypochlorite, were sown in each pot with three replications per genotype. Later on, thinning of pots was done at 3 leaf stage and three well-established seedlings were allowed to grow till maturity in each pot i.e., 18 plants per genotype were evaluated under two treatments (normal and drought). The day/night temperatures of the greenhouse were maintained at 30 °C/20 °C with air humidity ranging between 40 and 60%.

The drought stress during the reproductive stage was induced by withholding water application and maintaining moisture contents of the pots to 35% field capacity from heading to physiological maturity.

Filed capacity (100% water contents) of the soil in pots was considered after 24 h of full irrigation when the remainder of the water has been removed by the downward forces of gravity. This assumes that the water removed from the soil profile is only removed by gravity, not through the plants or evaporation. At this stage, the moisture contents of the pots were measured using Time Domain Reflectometry (Janik et al. 2021). Later on, the moisture content of pots was daily maintained at 35% of the TDR reading

Table 1 The detail of wheat lines used in this study

Entry nos.	Line code*/name
1	SBL 1
2	SBL 2
3	SBL 3
4	SBL 4
5	SBL 5
6	Pakistan 2013
7	SBL 7
8	SBL 8
9	SBL 9
10	SBL 10
11	SBL 11
12	Shahkar 2013
13	SBL 13
14	SBL 14
15	SBL 15
16	SBL 16
17	SBL 17
18	SBL 18
19	SBL 19
20	SBL 20
21	Faisalabad 2008
22	SBL 22
23	SBL 23
24	SBL 24
25	SBL 25
26	SBL 26
27	SBL 27
28	SBL 28
29	SBL 29
30	SBL 30
31	SBL 31
32	SBL 32
33	SBL 33
34	SBL 34
35	SBL 35
36	SBL 36
37	Ehsan 2016
38	SBL 38
39	SBL 39
40	SBL 40
41	SBL 41
42	SBL 42
43	SBL 43
44	Barani 2017
45	SBL 45
46	SBL 46

Table 1 (continued)

Entry nos.	Line code*/name
47	SBL 47
48	SBL 48
49	SBL 49
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 completely corresponds to their positions in all statistical analyses in this study

recorded at field capacity. When the moisture content of the pot declined to less than 30% of the total available water capacity of the soil, pots were re-watered with 300 ml of water to avoid permanent wilting.

The experiment was conducted in plastic pots of 8-L capacity filled with loam soil consisting of sand (60%), silt (25%), and clay (15%).

Data collection

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

The leaf chlorophyll contents were recorded using a SPAD-502 device (Minolta, Japan). Five readings of the chlorophyll content were measured per pot in 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 scissors from five random tillers per pot and the fresh weight (FW) of samples was taken instantly just after harvest. Then, samples were immediately placed in the icebox for relative water content (RWC) determination. The leaves were then submerged in distilled water for 24 h and the turgid weight (TW) of saturated leaves was recorded just after 24 h of submergence. The dry weight (DW) of the leaves was recorded after keeping samples in an oven at 80 °C for 24 h. The relative water content was determined using the following formula.

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

The Membrane Stability Index (STI) of leaf tissues was determined by recording the electrical conductivity (EC) of leaf leakages in double distilled

water at 40 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 the 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 a water bath at 40 °C for 30 min. (T1). The tubes were then kept at 15 °C for 24 h 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 min and then cooled to 25 °C and final conductivity (C2) was recorded (Deshmukh et al 1991).

The membrane stability index (MSI) was calculated as:

$$\text{MSI} = [1 - (C1/C2)] \times 100$$

The proline content was determined by using the acid-ninhydrin method developed by Bates et al (1973). Fresh leaf samples were harvested off the second top leaves from the drought-treated and control pots and immediately placed in the icebox. 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 the whole mixture was boiled for one hour at 100 °C in a water bath. Afterward, the test tubes were taken out of the 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 developed by the Bates et al (1973):

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

Pots were harvested at 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 the procedure described by the Pask et al (2012).

Genotypic coefficients of variation were calculated using the method proposed by Burton and DeVane (1953) as follows;

$$\text{Genotypic Coefficient of Variance(GCV)} = \sqrt{Vg/x} \times 100$$

Statistical analyses

The significance of treatment effects was calculated using the analysis of variance (ANOVA) technique, appropriate for two factors, in a completely randomized design (CRD) using statistical software Statistica 7 (Stat Soft, USA). The statistical software was used to compute summary statistics, the significance of differences between treatment means (Tukey's test), and Pearson's correlation analysis to develop the correlation matrix. The association between the performance of genotypes and phenotypic traits was further found using principal component analysis (PCA) using XLSTAT software (Adinsoft 2010). The PCA biplot was drawn between the 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 the R ver. 3.4.1 software package (R Core Team 2018).

Results

Analysis of variance (ANOVA), basic statistics, relative traits change, and coefficient of genetic variance

The analysis of variance results indicated significant ($P < 0.05$) effects of the environment (drought stress treatment), genotype, and environment x genotype interactions for most of the studied physio-agronomic traits (Table 2). The drought stress treatment (environment) significantly affected leaf chlorophyll (SPAD value), relative leaf water content (RLWC), membrane stability index (MSI), proline content (PC), kernels per spike (KPS), thousand kernel weight (TKW) and grain yield (GY). The ANOVA results also showed that there is significant variability among the studied genotypes with regard to physio-agronomic

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

Source of variation	DF	Physio-agronomic traits						
		SPAD	RLWC	MSI	PC	KPS	TKW	GY
Environment	1	13,101**	24,604.1**	41,550.9**	2927.44**	16,995.2**	9185.33**	140,660**
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

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

***Indicates significance at $P < 0.05$, $P < 0.01$ respectively and *ns* non-significant

traits under both normal and drought conditions. 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).

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 genotypes evaluated for drought tolerance at the reproductive stage is provided in Table 3.

The relative traits change (RTC) values of physio-agronomic traits were calculated as the 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 an inverse RTC value (-2.70) as it is increased under a drought stress environment in comparison to other

measured traits. The genetic coefficient variation (GCV) of physio-agronomic traits of the 50 wheat lines, tested under normal and drought-stressed environments, ranged from 9.30 to 30.6% (Table 3). The genetic variation of some traits i.e., MSI, RLWC, SPAD, TKW, and GY was > 20%, while the genetic variation of KPS ranged between 10 and 20%. The genetic variation coefficient for PC was < 10% (Table 3). These results indicated high genetic variation within the studied SBL under both normal and drought stress conditions.

The grain yields of 50 genotypes ranged from 43.5 to 53.4 g per pot under normal conditions with a mean value of 50 g (Table 2). The 25 SBLs showed higher while 20 lines showed lower GY than check varieties (cultivated commercial varieties) under normal conditions. The drought stress had high reducing effects on pooled means of grain yields of wheat lines. Under drought-stressed conditions, the GY of

Table 3 Mean values, range, relative traits change, and coefficient of genetic variance for the physiological and agronomic traits of the 50 synthetic backcross-derived wheat lines evaluated for drought tolerance at the reproductive stage

Physio-agronomic traits	Normal		Drought stress		RTC	CGV
	Mean \pm SD	Range	Mean \pm SD	Range		
SPAD value	50.4 \pm 2.1	46.8–53.2	37.2 \pm 6.4	29.5–50.1	0.23	23.3
RLWC (%)	81.6 \pm 2.3	76.5–84.7	63.5 \pm 9.5	44.3–79.3	0.28	30.6
MSI (%)	80.5 \pm 2.2	76.6–85.0	58.1 \pm 5.5	44.3–65.3	0.26	22.8
PC (μ mol per g FW)	2.30 \pm 0.6	0.5–3.9	8.5 \pm 1.6	4.4–12.4	-2.70	9.30
KPS (no.)	48.6 \pm 3.9	43.0–57.0	33.5 \pm 2.3	30.0–40.0	0.21	18.2
TKW (g)	34.7 \pm 1.2	33.0–37.0	23.6 \pm 2.8	19.0–30.0	0.32	22.7
GY (g/pot)	50.0 \pm 1.9	43.5–53.4	36.6 \pm 1.4	28.6–45.0	0.37	23.5

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 genotypes ranged from 28.6 to 45.3 g with a mean value of 36.6 g. The 12 SBLs indicated higher and 33 SBLs showed lower yield as compared to commercial checks (Fig. 1).

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 the correlation with physiological parameters like SPAD, RWC, and MSI was weakly positive and non-significant ($P>0.05$). The grain yield, under drought stress conditions, showed strong positive ($r>0.7$) and significant ($P<0.05$) correlations with TKW, KPS, SPAD, RLWC, MSI,

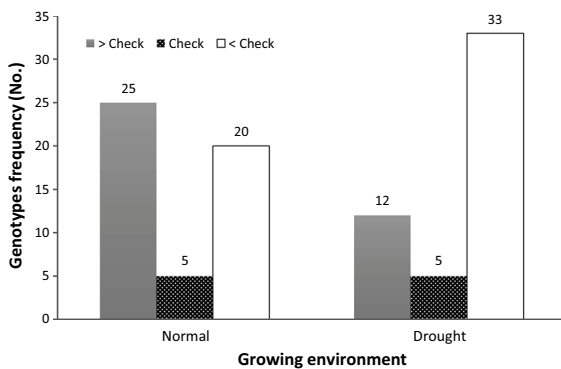


Fig. 1 Frequency distribution of grain yield of 45 SBL against 5 check commercial varieties under normal and drought stress conditions

and proline. 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 a 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 of all physio-agronomic traits. The variables like SPAD, MSI, RLWC, PC, KPS, TKW and GY had high positive loading into the first principal component. Under a normal growing environment, three principal components were important, recording 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

Table 4 Correlation coefficients (Pearson's) among agronomic and physiological traits of 50 synthetic backcross-derived wheat lines evaluated under drought-stressed and normal conditions

Traits	Normal						
	GY	KPS	TKW	SPAD	PC	MSI	RLWC
<i>Drought</i>							
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

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

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

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

Traits	Drought			Normal		
	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

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

proline and RLWC was noticed in the 3rd principal component.

The biplot analysis (Fig. 2) illustrates the associations between the different variables and genotypes with respective principal components for the normal (Fig. 2a) and drought stress conditions (Fig. 2b). The acute angles (<90°) between dimension vectors in

a similar direction showed a positive correlation of the variable traits in terms of describing genotypes. The genotypes outrivalling 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 a positive correlation (<90°) under drought

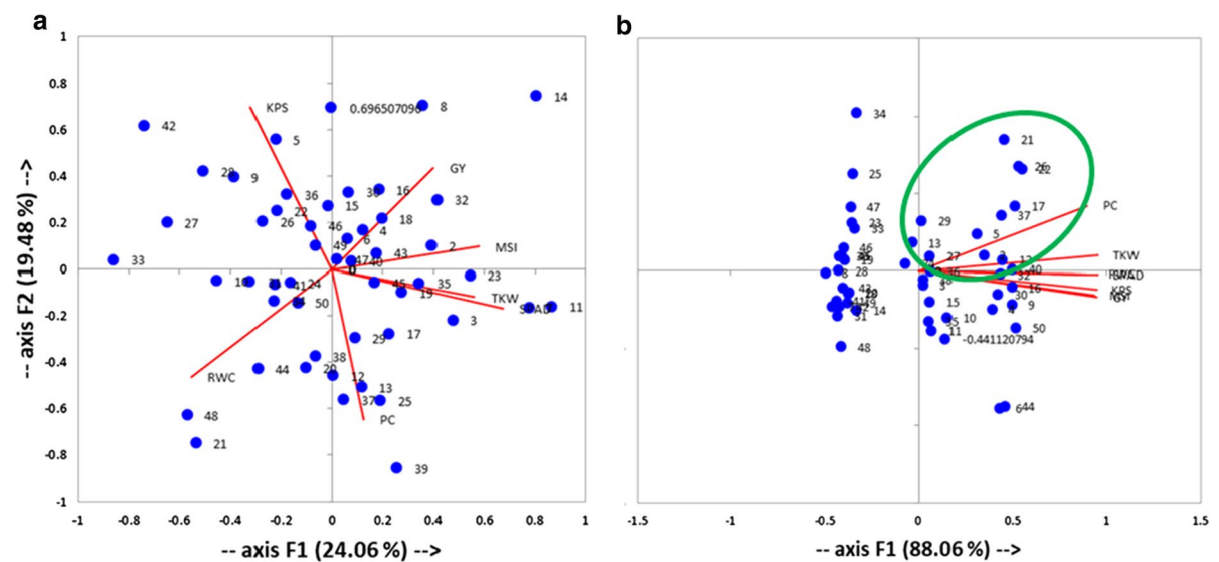


Fig. 2 Principal component analysis Biplot. **a** PCA biplot for traits and genotypes studied under normal and **b** PCA biplot for traits and genotypes studied under drought stress treatment.

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

stress (Fig. 2b). However, under normal conditions, GY was only positively correlated with TKW, KPS, SPAD, and MSI (Fig. 2a). Moreover, PCA biplot analysis helped to group 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 on the positive side of the first principal component (Fig. 2b) and are encircled green. These genotypes excelling in overall productivity over check varieties were contributed mostly by high TKW, RLWC, and optimum values for other physio-agronomic traits as well, nevertheless, the genotypes were more scattered on both sides of the two principal components under normal condition (Fig. 2a).

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 were 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 color 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 a negative Z-score (dark blue color) consisted of 21 SBLs which showed less mean yield attributes and were 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. Group 2, comprised of 17 SBLs (12 SBL and 5 checks) with a strong positive Z-score (dark yellow color) for yield contributing traits is considered a drought tolerant group. It includes 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 a weak positive Z-score (light yellow color) and was accepted as a moderate drought tolerant group (Fig. 3).

Discussion

The development of climate-resilient wheat cultivars is the prime objective of wheat breeders, and they are persistently improving the germplasm

of crop plants for drought tolerance by selection methods and genetic modification (Ehlers and Goss 2003; Fleury et al. 2010).

The wild relatives of wheat are being utilized by the wheat breeders as an important source of drought tolerance (Pour-Aboughadareh et al. 2017c), exploring synthetic backcross-derived wheat lines for drought tolerance potential is a substantial approach to widening wheat genetic diversity for advanced wheat breeding programs. The SBL used in this study showed highly significant variation for all recorded physiological and agronomic traits indicating that it seems to be a rich source of genetic diversity for the breeding objective of drought tolerance. The observed significant effects of the environment, genotype, and environment x genotype interactions for most of the phenotypic traits were expected. Better growth and grain yield of certain SBLs than local checks were linked with their superior scores of some physiological and agronomic yield contributing traits, especially grain weight is most differentiating between genotypes. The results showed a strong contribution of these physio-agronomic traits towards grain yield under moisture-stressed conditions. 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 grain yield with relative leaf water contents, leaf chlorophyll, membrane stability index, proline contents, kernels per spike, and thousand kernel weight under water stress conditions indicate the direct impact of these yield contributing components to grain yield. Dodig et al (2012) and Sareen et al (2014) also inferred related findings after screening wheat accessions and landraces for drought tolerance potential. It is also reported that the tolerant genotypes 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) and by a more efficient and deeper root system (Nehe et al. 2021). The results imply that selection for these physio-agronomic traits could significantly improve the yields under drought and these parameters should be used as important target traits while screening genotypes for drought tolerance.

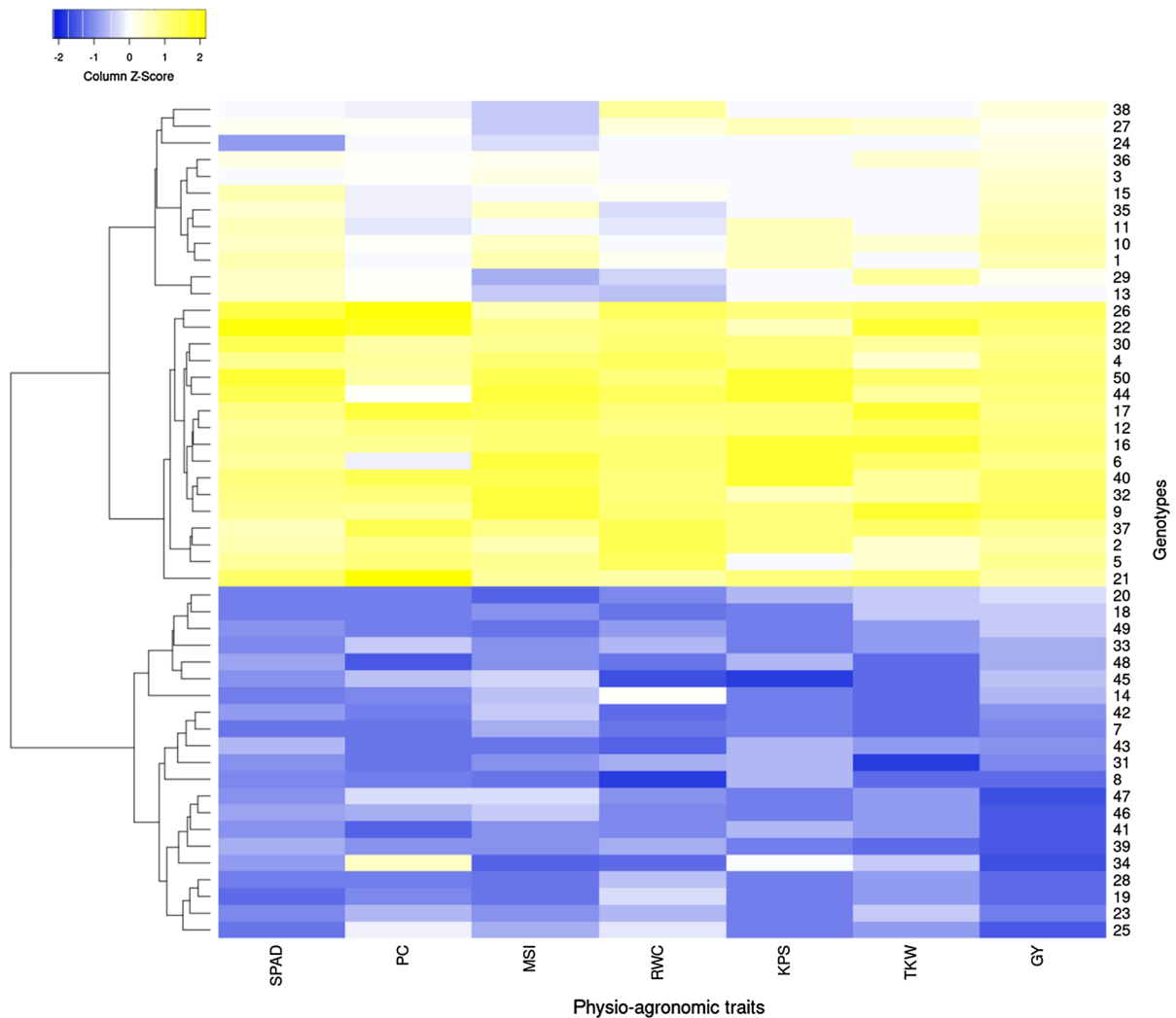


Fig. 3 Heatmap and hierarchical clustering (ward's method) of the mean values of physio-agronomic traits of the wheat genotypes under normal and drought stressed conditions. The similar z-score color and the distance between clusters show

the similarity of the genotypes. *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 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 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 indicates that they are influential physiological traits and

have a direct impact on each other. They might simultaneously be considered as selection criteria for the 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 cells primarily result in a stomata closure, as the plant tries to reduce water loss from plant tissues, resulting in a decline in the availability of CO₂ and consequently a reduction in photosynthetic efficiency (Lawlor and Cornic 2002). The drought stress can also induce changes in other bio-physiological mechanisms like chlorophyll synthesis and changes in the structure and function of chloroplasts, disorder in the accumulation of assimilates (Medrano et al. 2002; Flexas and Medrano 2002).

In our study, under normal growing conditions, the SBL maintains a higher leaf water status and chlorophyll to have the 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 are enhanced which might be an indicator of the osmotic adjustment to maintain the 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 based on grain yield and associated yield contributing phenotypic traits in both drought-stressed and normal environments. The outputs of PCA and univariate statistics allowed to classify the groups of similarity and indicated that the drought tolerance potential of some similar genotypes has common intrinsic genotypic traits. In drought tolerant SBLs, grain yield reduction was significantly lower than 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 cytoplasmic 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 the membrane stability index measured of electrolyte leakage from fresh plant tissues is an effective tool to assess the 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.

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 for drought tolerance and found the existence of a wide range of genotypic variability among tested genotypes. The findings contribute to identifying 12 drought-tolerant SBL (04, 05, 09, 16, 17, 21, 26, 30, 32, 37, 40, and 50) which might have intrinsic bio-physiological mechanisms to tolerate drought stress and can be used in the breeding program to drought-tolerant cultivars. The physiological traits determination like Leaf chlorophyll and 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 levels 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 of the importance of synthetic backcross-derived wheat lines as valuable genetic resources for widening the gene pool of bread wheat and to be incorporated into the wheat breeding program to enhance the drought resilience adaptability of cultivated wheat.

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Author's contribution 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 of the research. S. Waqar assisted in data collection. I. Hussain served as a research supervisor and 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.

Declarations

Conflict of interest The authors have no conflicts of interest or competing interests.

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