



### Full Length Article

## Terminal Drought Stress Adaptability in Synthetic-Derived Bread Wheat is explained by Alleles of Major Adaptability Genes and Superior Phenology

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### Abstract

Establishing the agronomic superiority of wheat germplasm derived from crop-wild introgressions under field stress conditions is of prime importance in breeding. Comparative performance between synthetic-derived bread wheat (SBW) and conventional bread wheat (CBW) under terminal drought stress condition was evaluated in this study. Evaluations were carried out under well-watered and drought stress conditions for two consecutive years at pre-anthesis stage of crop. Based on drought susceptibility index (DSI) values, the SBW genotypes SBW6, SBW8, SBW10, SBW13, SBW22, SBW23 and SBW24 had low values than the check varieties under drought stress. The genotypes exhibited narrow range of flowering time, however SBW with photoperiod insensitive (*Ppd-D1a*) and wild-type *Rht-B1a/Rht-D1a* alleles have clear yield advantage over CBW under terminal drought stress. Likewise, winter-type *vrn-A1* and *vrn-D1* alleles slightly increased heading time and enhanced yield in SBW. SSR based population structure inferred from Bayesian analysis and principal component analysis (PCA) dissected the population into 2 sub-groups, with most of the SBW in one sub-population and slight admixture between SBW and CBW. In conclusion, these results indicate the agronomic superiority of SBW under terminal drought stress is likely through retention of favorable alleles in SBW sub-population. Low DSI (DSI<1) values can be used as a selection criterion for increasing yields under terminal drought conditions. © 2018 Friends Science Publishers

**Keywords:** Drought susceptibility index (DSI); Simple sequence repeats (SSRs); *Ppd-1*, *Rht-1*; *Vrn-1*; Synthetic-derived bread wheat (SBW)

### Introduction

Being the world's most widely grown cereal crop, wheat (*Triticum aestivum* L.) is cultivated on about 21.8% of the present agricultural land. High yielding wheat varieties which are tolerant to biotic and abiotic stresses fulfills a large demand for wheat consumption especially in developing world. Hence, development of environment resilient wheat cultivars particularly tolerant to drought stress, are essential to get sustainable wheat production for projected global demands (Semenov and Halford, 2009). Harnessing new alleles from the wild gene pools of wheat through interspecific and intergeneric hybridization are crucial to achieve this goal (Mujeeb-Kazi *et al.*, 2013). *Aegilops tauschii*, D genome donor to bread wheat, is an important reservoir of novel alleles preferentially available for bread wheat genetic improvement for various biotic (Mujeeb-Kazi *et al.*, 2008), and abiotic stresses (Trethowan and Mujeeb-

Kazi, 2008). This novel diversity can be introduced to wheat through interspecific hybridization of durum wheat and wild accessions of *Ae. tauschii* where the reconstituted amphiploids are known as synthetic hexaploids wheats (SHWs) (Ogonnaya *et al.*, 2013). SHWs have been extensively characterized for their tolerance to drought stress (Trethowan and Mujeeb-Kazi, 2008), however their derived advances lines are less emphasized to assess their contribution for drought tolerance. Recently, McIntyre *et al.* (2014) characterized synthetic derived advances lines and assessed the preferential retention of chromosomal regions associated with superior agronomic traits in SHWs.

In drought, agronomic stability is mainly dependent upon these keys factors i.e., minimizing loss of water, improvement in the uptake of water which can be done by either reduction in the rate of transpiration or development of deeper roots, adjusting osmotic status or by reducing total leaf area (Farooq *et al.*, 2009; Hu and Xiong, 2014).

The flowering time also play a decisive role and can be modulated to escape drought, a response to accelerate flowering to avoid drought stress (Farooq *et al.*, 2014; Kazan and Lyons, 2016). Therefore, the genetic loci underlying these traits are helpful to understand the basis of drought tolerance in germplasm. For example, photoperiod response (*Ppd-1*) and vernalization (*Vrn-1*) loci largely determine the flowering time and are important to screen to assess the confounding effects of flowering time in drought adaptability. The allelic effects of these adaptability genes (photoperiod response (*Ppd-1*) and vernalization (*Vrn-1*) are not well studied in synthetic derived wheat under drought stress conditions. Therefore, phenotypic comparison between synthetic-derived bread wheat (SBW) and conventional bread wheat (CBW) under terminal drought stress in field conditions and allelic effect of adaptability genes were performed in this study. The objectives of the current work were, 1) to investigate and compare variations in the expression of agronomic traits under terminal drought conditions among synthetic derived wheats, conventional wheats and check cultivars; 2) allelic effects of genes underpin wheat adaptability under terminal drought stress, and 3) to analyze the genetic variation between conventional and synthetic derived bread wheats based on SSR markers.

## Materials and Methods

The germplasm consisted of 26 synthetic-derived bread wheats (SBW1-SBW26), 24 conventional bread wheats (CBW1-CBW24) and five local cultivars i.e., C1 (Inquilab-91), C2 (Seher-2006), C3 (Chakwal-50), C4 (NR-372) and C5 (Wafaq-2001). SBWs were produced by crossing primary synthetics with drought susceptible bread wheat cultivars. The pedigrees of the experimental genotypes are given in Table 1.

Field experiments were carried out during two consecutive seasons at National Agriculture Research Center, (NARC), Islamabad (33°43'N 73°04'E). Deep and well-drained soil of Gujranwala type is present at the experimental site which shares a portion of Potohar upland (Location 6; Rashid *et al.*, 1994). Soil particles are smooth and fine textured having pH of 8.1. It is non-saline, somewhat calcareous with an electrical conductance of 0.24 dS m<sup>-1</sup>. Randomized complete block design (RCBD) was used for experimental layout in both control (field) and stressed (tunnel) conditions. Each variety with three replications was sown in 2 meters long row which formed an experimental unit. A 30 cm inter-row spacing was maintained between each row. This experiment is comprised of two different water regimes i.e. control and tunnel. Varieties under control treatment are sown in open field with standard irrigation while varieties under stressed treatment are sown in tunnel which is covered with a shelter to avoid precipitation. Boundary of tunnel was surrounded by a 1 m ditch to avoid water seepage from rain. 30 viable seeds were sown in each line with the help of a small-plot

grain drill. In order to maintain a uniform stand different seeds with variable kernel weight were selected. Wheat was planted on November 19, 2013 and November 21, 2014 during first and second year of experimentation, and was harvested in mid-April during both years. Drought treated plots were covered with polyethylene sheets supported on iron frames of the tunnel at the end of tillering to prevent it from precipitation. Water was withheld till flowering was completed. The crop growth stages were determined using the Zadoks scale (Zadoks *et al.*, 1974). Soil moisture was monitored during this period with the help of TDR soil moisture meter (Spectrum Technologies, Illinois, USA). Drought stress was maintained at 12.5% soil moisture (evident from wilting symptoms of the plants) till post-anthesis stage. Three irrigations were given to plants in open field (control), and soil moisture was maintained at field capacity (100%) until harvest.

Data were recorded for days to flowering by counting number of days from sowing to when 50% of the plants reached flowering stage. At Z96 stage (physiological maturity) (Zadoks *et al.*, 1974), data on spikes per plant (SPP), plant height (PH), spike length (SPL), days to physiological maturity (PM) were recorded. Spikes per plant (SPP) were counted from randomly selected plants for each variety, plant height (PH) was measured from the base of the plant to the spike tip excluding awns with meter rod, spike length (SPL) was measured from the point where the spike originates to the end of last spikelet excluding awns and days were counted from sowing to physiological maturity to calculate days to physiological maturity (PM). After harvesting, grain yield per m<sup>2</sup> (GY) and thousand grain weight (TGW) were recorded. Drought susceptibility index (DSI) was also measured according to the following equation developed by Fischer and Maurer, (1978):

$$DSI = (1 - Y/Y_p)/(1 - X/X_p)$$

Where Y<sub>p</sub> = Yield of variety potential under control, Y = Yield of variety under drought stress, X<sub>p</sub> = Mean of all test varieties under control, X = Mean yield of all test varieties under drought.

## Statistical Analysis

Analysis of variance was performed using SAS version 11. Values were set according to randomized model for all recorded phenological data in both drought and control environments. Fisher's LSD (least significant difference) was done to find statistical differences among treatment means for all phenotypic traits. STATISTICA software (version 7.0) was to find Pearson's correlation for determination of relationship between variables. The allelic effects on two germplasm groups were compared based on student's t-test.

## Genotyping for SSRs and Functional Genes

Selection of simple sequence repeats (SSRs) was done on

the basis of allele numbers, the quality of amplified product, and polymorphism. According to the sequence information of primers provided by Roder *et al.* (1998), total 101 SSRs were selected. SSR markers were allocated positions on the chromosomes by using consensus map of Somers *et al.* (2004). According to this, 25 SSR markers fit to A genome, 29 to B genome, and 47 to D genome. Protocol for DNA extraction and PCR analysis have been described earlier (Ali *et al.*, 2015). For allelic variations of *Ppd-D1*, *Vrn-D1*, *Vrn-B1*, *Vrn-A1*, *Rht-B1* and *Rht-D1*, allele-specific KASP markers were used according to Rasheed *et al.* (2016).

Power Marker software (Version 3.25) was used to calculate gene diversity of SSRs, polymorphic information content (PIC), allele number, and gene frequency (Liu and Muse, 2005). As suggested by Brescaglio and Sorrells, (2006), rare alleles (allelic frequency < 5%) were considered as missing data.

### Population Structure and Diversity Analysis

Population structure was evaluated using processed genotypic data of SSR markers using STRUCTURE software 2.3.3 which implements a model-based Bayesian cluster analysis (Pritchard *et al.*, 2000). These SSR markers are spread over whole genome of wheat.

Associated allelic frequencies was used to apply admixture model (Falush *et al.*, 2003). Genetic structure was tested using burn in phase of  $10^5$  iterations and MCMS (Markov chain Monte Carlo) periods of  $10^5$ . Consistency of results for each run was compared along independent runs by performing 10 runs for K (K=number of subpopulations) values. A hypothetical subgroup was assigned which ranged from 2 to 20. Representative run was selected based on the data with highest subsequent probability. Defining subgroups does not require any previous knowledge about source or phenotypic information. According to Evanno *et al.* (2005),  $\Delta K$  was plotted against K to determine the number of possible sub-populations in data. It was further confirmed by making a plot between  $\Delta K$  and subclasses K.

### Results

Genotypes and treatments (control and terminal drought) exhibited significant differences for the traits studied. Data were analyzed statistically and the differences were visualized using Fischer's LSD (Table 2). Analysis of variance for all phenological traits showed significant difference among genotypes, year, treatment and their interaction with some exceptions (Table 3). Genotype interaction with treatment and years also differed significantly for all traits except SPP. Statistical analysis of data and visualization of differences was done by Fischer's LSD (Table 2). Genotype interaction with treatment and years also differed significantly for all traits except SPP. There were significant differences among all traits in both control and stress (terminal drought). It was rendered that in

terminal drought stress condition that there was an obvious reduction in most of the phenotypic traits across both years. Reduction in PH was 12%, in GS was 14.3%, in DF was 7.1%, in SL was 10.1%, in TGW was 8.9%, in PM was 5.6% and similarly SP was reduced to 15.8%. Range of CV (coefficient of variation) varies from 1.3% (TGW) to 15.8% (GY). TGW was found to be the most consistent with respect to CV%. The other consistent traits were PH, DF, and PM having CV of 2.6%, 3.5%, and 3.3% respectively.

### Effect of Genotypes on Agronomic Traits

The phenotypic performance of three germplasm groups were compared (Table 4) SBW lines were significantly superior to the corresponding checks for maximum traits in both years. SBW in terms of PH, TGW, GS, and SPL showed superior performance as compared to check cultivars. In SBW, there was 10.2%, 21.1%, 32.5% and 4.9% increase in PH, TGW, GS, and SPL respectively in comparison to check cultivars. In correspondence to check cultivars, TGW also showed early DF and PM i.e., 2.4% and 2.0% respectively. However, there was 4.5% increase in SPL in check cultivars than SBW. Almost similar findings were observed in control conditions as well. Comparing CBW and SBW, there was superior performance of SBW w.r.t. PH (2.0%), TGW (3.6%), and SPP (11.4%) showing 1.6% earliness in PM and 2.6% in DF. Almost similar findings were taken in this group for control.

There were eight synthetic derived lines (SBW4, SBW5, SBW6, SBW8, SBW10, SBW13, SBW22 and SBW26) having significantly higher GY as compared to the check cultivars (C3, C4 and C5). Moreover, four of the best SBW genotypes for GY (SBW8, SBW10, SBW13 and SBW24) had higher TGW than the check cultivars. Seven SBW genotypes (SBW6, SBW8, SBW10, SBW13, SBW22, SBW23 and SBW24) among the top drought tolerant genotypes showed lowest DSI score. These genotypes were also better performing in early flowering, high SPP, SPL, TGW and GY. However, few genotypes from CBW (CBW3, CBW5, CBW11 and CBW24) and also from local check cultivars (C3 and C5) were also among the top drought tolerant genotypes. On the other hand, the drought susceptible lines based on highest DSI score were those possessing high DF and having lowest SPP, SPL, TGW and GY. Positive correlation ( $r=0.32$ ) has been seen among DSI and DF and DSI was found to be in significant negative correlation with SPP ( $r=-0.65$ ), SPL ( $r=-0.33$ ), TGW ( $r=-0.37$ ) and GY ( $r=-0.64$ ) (Table 4).

### Drought Susceptibility Index

DSI reflects the comparison of cultivars yield potential ( $Y_p$ ) and its yield in stress environment. Based upon DSI values, classification of genotypes was done; (a) genotypes having  $DSI \leq 0.5$  were classified under extraordinarily drought tolerant, (b) genotypes having  $DSI > 0.5$  to 1.0 were classified under relatively drought tolerant, and (c)

**Table 1:** Pedigree information of the germplasm used in this study

Group	Sr. No.	Pedigree
Conventional bread wheats	CBW1	EXCALIBUR/WBLL1
	CBW2	TEV2/FRET2
	CBW3	CETTIA/WBLL1
	CBW4	TC870344/GU1//TEMPORALERA M 87/AGR/3/WBLL1
	CBW5	PVN/3/PRL/SARA//TSI/VEE#5/4/WBLL1
	CBW6	BAV92/3/PRL/SARA//TSI/VEE#5/4/WBLL1
	CBW7	PASTOR/3/URES/JUN//KAUZ/4/WBLL1
	CBW8	1455/2*PASTOR
	CBW9	AUS 4930.7/2*PASTOR
	CBW10	KRICHAUFF/2*PASTOR
	CBW11	ATTILA/WBLL1
	CBW12	TUI//2*SUNCO/SA1166/3/TUI/4/FINSI
	CBW13	HXL7573/2*BAU//WBLL1
	CBW14	GPO8 KAZAKSTAN 6 WM98-99/4/KAUZ//ALTAR 84/AOS/3/ KAUZ/5//KAUZ//ALTAR 84/ AOS/3/KAUZ
	CBW15	RABE/2*MO88/3/CAZO/KAUZ//KAUZ
	CBW16	MILAN/BERKUT
	CBW17	PRINIA/BERKUT
	CBW18	QT8343//PASTOR*2/OPATA
	CBW19	JARU/3/URES/JUN//KAUZ/4/URES/JUN/KAUZ
	CBW20	TAN//TEMPORALERA M 87/AGR/3/NG8319//SHA4/LIRA
	CBW21	JNRB.5/PIFED
	CBW22	JNRB.5/PIFED
	CBW23	KAUZ*2/BOW//KAUZ/3/W98.6.38
	CBW24	SARA/THB//VEE/3/VEE/PJN//2*KAUZ
Synthetic-derived bread wheats	SBW1	PASTOR/3/KAUZ*2/OPATA//KAUZ/4/CHEN/AE.SQ//2*OPATA
	SBW2	PAM94/3/ALTAR 84/AEGILOPS SQUARROSA(TAUS)//OPATA/4/PASTOR
	SBW3	T. DICOCCON PI94625/AE.SQUARROSA (372)//FRET2/3/2*WBLL1
	SBW4	T.DICOCCON PI225332/AE.SQUARROSA (895)//WBLL1/3/2*WBLL1
	SBW5	FRET2/3/CHEN/AE.SQ//2*OPATA
	SBW6	FRET2/3/CHEN/AE.SQ//2*OPATA
	SBW7	FRET2/3/CHEN/AE.SQ//2*OPATA
	SBW8	DOY1/AE.SQUARROSA(333)/3/PRL/VEE#6//CHOIX/4/HAHN/PRL//CLMS/3/HAHN/PRL
	SBW9	ARLIN_1/AE.SQUARROSA (1017)//ATTILA/3/ATTILA*2/M10 (MUTATED C-306)
	SBW10	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (882)/6/ATTILA/10/
	SBW11	URES/JUN//KAUZ/3/ALTAR 84/AE. SQ//2*OPATA
	SBW12	CROC_1/AE.SQUARROSA (205)//KAUZ/3/ENEIDA/4/FINSI
	SBW13	FILIN/IRENA/5/CNDO/R143//ENTE/MEXI 2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/6/...
	SBW14	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/OCI/5/
	SBW15	FILIN/IRENA/5/CNDO/R143//ENTE/MEXI 2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/6/BERKUT
	SBW16	FILIN/IRENA/5/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/6/BERKUT
	SBW17	FILIN/3/CROC_1/AE. SQUARROSA (205)//KAUZ/4/FILIN/5/VEE/MJI//2* TUI/3/PASTOR
	SBW18	FILIN/3/CROC_1/AE. SQUARROSA (205)//KAUZ/4/FILIN/5/VEE/MJI//2* TUI/3/PASTOR
	SBW19	CROC_1/AE. SQUARROSA (205)//KAUZ/3/PRL/SARA//TSI/VEE#5
	SBW20	CROC_1/AE.SQUARROSA (205)//KAUZ/3/2*PJN/BOW//OPATA
	SBW21	BABAX/3/PRL/SARA//TSI/VEE#5/4/CROC_1/AE.SQUARROSA (224)//2*OPATA
	SBW22	CROC_1/AE.SQUARROSA (205)//BORL95/3/KENNEDY
	SBW23	CNDO/R143//ENTE/MEXI_2/3/ AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*JANZ
	SBW24	CROC_1/AE.SQUARROSA (205)//BORL95/3/KENNEDY
	SBW25	CROC_1/AE.SQUARROSA (205)//BORL95/3/KENNEDY
	SBW26	CHEN/AEGILOPS SQUARROSA (TAUS)//BCN/3/BAV92
Local cultivars	C1	Inqilab 91
	C2	Seher 2006
	C3	Chakwal 50
	C4	NR372
	C5	Wafaq 2001

genotypes having DSI >1.0 were classified under drought susceptible. According to this classification, SBW13 and SBW10 were classified as drought tolerant with DSI of 0.35 and 0.49 respectively. Inqilab-91 with DSI of 1.53 and SBW15 with DSI of 1.56 were regarded as the most drought susceptible genotypes. In this study, 22 genotypes were found to have DSI < 1.0. As compared to rainfed check cultivars, among these 22 genotypes SBW6, SBW8, SBW10, SBW13, SBW22, SBW23 and SBW24 possessed

lowest DSI values. Narrowing down the selection criteria SBW8, SBW10, SBW13 and SBW24 performed best for DSI and as well as for TGW and GY.

### Genetic Diversity and Population Structure

In total, 525 alleles were observed over 101 SSR loci with an average of 5.19 alleles per locus. The number of alleles ranged from 2 to 14 alleles per locus.

**Table 2:** Comparison of means among check cultivar, synthetic derived (SBW) and conventional (CBW) bread wheat under irrigated and drought stress. Fisher's protected LSD values given for  $P < 0.05$ 

Treat.	Germplasm	PH	DF	PM	SP	SL	GS	TGW	GY
Irrigated	SBW	95.24	114.00	144.02	12.90	12.59	52.28	43.97	2250
	CBW	93.92	116.85	146.15	12.24	12.35	53.35	43.16	2098.5
	Check	84.47	116.10	146.10	13.53	11.35	41.10	36.27	1507.3
	SBW	83.86	105.75	135.75	11.11	11.20	44.90	40.55	1553.2
	CBW	82.22	108.58	137.95	9.97	11.14	45.84	38.93	1328.2
Stress	Check	76.07	108.30	138.50	11.63	10.68	34.13	32.47	979.5
	LSD for Treat	1.10***	0.83***	0.89***	0.47***	0.27***	1.45***	0.72***	99.1***
	LSD for Group	1.64***	1.25***	1.31***	0.60***	0.34**	1.81***	0.87***	232.4 ***
	LSD for Treat*Group	1.78***	1.39***	1.51***	0.80***	0.46***	2.36***	1.11***	232.5 NS

Where, \*\* and \*\*\* significant at the 0.01 and 0.001 probability level

PH, plant height (cm); DF, days to flowering; PM, days to physiological maturity; SPP, spikes per plant; SPL, spike length (cm); GS, grains per spike; TGW, thousand grain weight (g) and GYP, grain yield per m<sup>2</sup> (g)

**Table 3:** Analysis of variance for the phenological traits under irrigated and drought stress during for the studied wheat germplasm (including 5 check cultivars)

Source	d.f	MS	F	R <sup>2</sup>	P ≥ F	MS	F	R <sup>2</sup>	P ≥ F	MS	F	R <sup>2</sup>	P ≥ F
Plant height													
Year	1	3706.24	702.89	6.75	0.000	670.04	44.22	2.20	0.000	2045.82	93.74	6.18	0.000
Treat.	1	20877.10	3959.50	38.05	0.000	11152.10	736.05	36.64	0.000	11029.10	505.36	33.30	0.000
Gen.	54	382.31	72.50	37.62	0.000	147.68	9.75	26.21	0.000	107.53	4.93	17.53	0.000
Year*Treat	1	35.01	6.64	0.06	0.010	6.40	0.42	0.02	0.523	155.16	7.11	0.47	0.008
Year*Gen.	54	95.06	18.03	9.35	0.000	28.69	1.89	5.09	0.000	27.27	1.25	4.45	0.120
Treat*Gen.	54	37.38	7.09	3.68	0.000	27.71	1.83	4.92	0.001	32.39	1.48	5.28	0.018
Year*Treat*Gen.	54	2.53	0.48	0.25	0.999	16.98	1.12	3.01	0.267	23.28	1.07	3.80	0.355
Error	440	5.00				15.00				22.00			
Days to flowering													
Year	1	565.64	237.94	8.23	0.000	268.68	241.28	11.86	0.000	1485.00	180.03	2.17	0.000
Treat.	1	666.01	280.16	9.69	0.000	255.32	229.29	11.27	0.000	9035.36	1095.40	13.19	0.000
Gen.	54	79.34	33.38	62.36	0.000	20.45	18.37	48.75	0.000	976.70	118.41	76.98	0.000
Year*Treat	1	0.34	0.14	0.00	0.707	8.09	7.27	0.36	0.007	102.42	12.42	0.15	0.001
Year*Gen.	54	2.84	1.20	2.24	0.170	0.25	0.22	0.59	1.000	10.08	1.22	0.79	0.144
Treat*Gen.	54	2.42	1.02	1.91	0.441	2.08	1.87	4.95	0.000	13.34	1.62	1.05	0.005
Year*Treat*Gen.	54	0.44	0.19	0.35	1.000	0.25	0.23	0.60	1.000	4.68	0.57	0.37	0.994
Error	440	2.38				1.11				8.00		5.30	
Spikes per plant													
Year	1	227.83	854.59	1.33	0.000	1407.60	102.84	2.07	0.000				
Treat.	1	2611.00	470.20	15.32	0.000	14945.90	1091.89	22.00	0.000				
Gen.	54	257.67	966.53	81.49	0.000	787.80	57.55	62.62	0.000				
Year*Treat	1	4.75	17.82	0.03	0.000	4.80	0.35	0.01	0.553				
Year*Gen.	54	0.57	2.15	0.18	0.000	17.70	1.29	1.40	0.090				
Treat*Gen.	54	2.60	9.76	0.82	0.000	34.50	2.52	2.74	0.000				
Year*Treat*Gen.	54	0.47	1.76	0.15	0.001	3.60	0.26	0.29	1.000				
Error	440	0.27				13.7							
Spike length													
Year	1	227.83	854.59	1.33	0.000	1407.60	102.84	2.07	0.000				
Treat.	1	2611.00	470.20	15.32	0.000	14945.90	1091.89	22.00	0.000				
Gen.	54	257.67	966.53	81.49	0.000	787.80	57.55	62.62	0.000				
Year*Treat	1	4.75	17.82	0.03	0.000	4.80	0.35	0.01	0.553				
Year*Gen.	54	0.57	2.15	0.18	0.000	17.70	1.29	1.40	0.090				
Treat*Gen.	54	2.60	9.76	0.82	0.000	34.50	2.52	2.74	0.000				
Year*Treat*Gen.	54	0.47	1.76	0.15	0.001	3.60	0.26	0.29	1.000				
Error	440	0.27				13.7							
Grains per spike													
Year	1	227.83	854.59	1.33	0.000	1407.60	102.84	2.07	0.000				
Treat.	1	2611.00	470.20	15.32	0.000	14945.90	1091.89	22.00	0.000				
Gen.	54	257.67	966.53	81.49	0.000	787.80	57.55	62.62	0.000				
Year*Treat	1	4.75	17.82	0.03	0.000	4.80	0.35	0.01	0.553				
Year*Gen.	54	0.57	2.15	0.18	0.000	17.70	1.29	1.40	0.090				
Treat*Gen.	54	2.60	9.76	0.82	0.000	34.50	2.52	2.74	0.000				
Year*Treat*Gen.	54	0.47	1.76	0.15	0.001	3.60	0.26	0.29	1.000				
Error	440	0.27				13.7							

Lowest genetic diversity among markers was seen in *Xbarc42-3DS* i.e., 22% while the highest was seen in *Xwmc718-4AL*, *Xgwm698-7AL*, and *Xgwm484-2DS* i.e., 86%, 85%, and 84%. Among all markers, Average genetic diversity was 57%. Mean of polymorphic information content (PIC) was 0.52 and it ranged from 0.20 (*Xbarc42-3DS*) to 0.84 (*Xwmc718-4AL*). The frequency of major alleles varied from 0.22 to 0.88. When comparison was made among groups, the level of genetic diversity was the highest in CBW (0.56) and SBW (0.55) while it was lowest in local cultivars (0.42). Population structure matrix (Q) was recorded by running structure at K=2 (Fig. 2) where the highest value of  $\Delta K$  occurred demonstrating its maximum likelihood as given in Fig. 2b. Similar results were obtained by principal component analysis, where two clusters

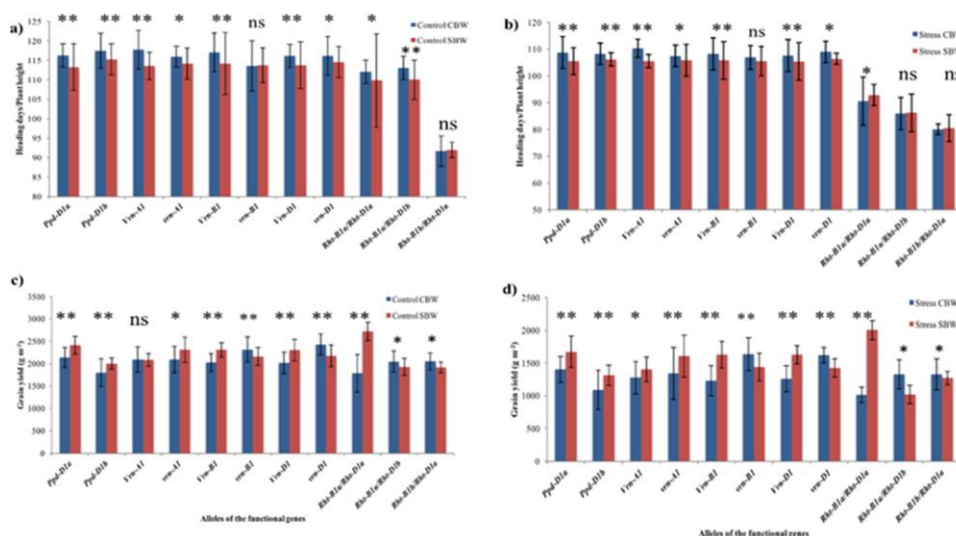
obtained in PC1 versus PC2, explaining 24% of the total variability, which is in concordance to the STRUCTURE.

### Allelic Effects of Functional Genes for Agronomic Traits

The allelic frequencies for all functional genes were studied. One of the wild-type either *Rht-B1a* or *Rht-D1a* were present in 19 CBW and 13 SBW genotypes (Fig. 1). There were significant differences between control and drought stress conditions in all allelic groups. Although there were no significant differences in heading time between *Ppd-D1a* and *Ppd-D1b* genotypes, however GY differed significantly between both allelic groups. Genotypes with insensitive type *Ppd-D1a* alleles were superior in GY, while SBW had significantly superior GY than CBW. Similar trend was

**Table 4:** Pearson coefficient of correlation (r) and associated probabilities (\*, \*\* and \*\*\* for  $P \leq 0.05$ ,  $\leq 0.01$ ,  $\leq 0.001$  respectively) between measured phenological traits using means of each line (n=55). Lower triangle represents trait correlations separately for irrigated and stress while upper triangle shows correlations of traits combined for irrigated and stress

		PH	DF	PM	SPP	SPL	GS	TGW	GY	DSI
PH	Irrigated		-0.32*	-0.24	0.28*	0.52***	0.31*	0.42**	0.47***	-0.14
	Stress									
DF	Irrigated	-0.17		0.67***	-0.19	-0.17	-0.09	-0.32*	-0.27*	0.32*
	Stress	-0.31*								
PM	Irrigated	-0.16	0.92***		-0.02	-0.05	-0.02	-0.27*	-0.11	0.20
	Stress	-0.13	0.33*							
SPP	Irrigated	0.21	-0.25	-0.16		0.32*	0.13	0.18	0.77***	-0.65***
	Stress	0.35**	-0.07	0.14						
SPL	Irrigated	0.49***	-0.19	-0.12	0.28*		0.54***	0.31*	0.57***	-0.33*
	Stress	0.55***	-0.06	0.12	0.40**					
GS	Irrigated	0.30*	-0.03	0.03	0.11	0.55***		0.07	0.61***	-0.21
	Stress	0.31*	-0.11	-0.01	0.29*	0.51***				
TGW	Irrigated	0.29*	-0.29*	-0.30*	0.07	0.29*	0.03		0.50***	-0.37**
	Stress	0.52***	-0.24	-0.15	0.23	0.37**	0.08			
GY	Irrigated	0.40**	-0.20	-0.18	0.75***	0.58***	0.63***	0.40**		-0.64***
	Stress	0.53***	-0.27*	0.02	0.80***	0.59***	0.62***	0.49***		



**Fig. 1:** Allelic effects of major adaptability genes on morphological traits **a)** Allelic effects of major adaptability genes on heading time and plant height in control and **b)** drought stress conditions, the values for *Rht* genes represent plant height (cm), **c)** Allelic effects of major adaptability genes on grain yield in control and **d)** drought stress conditions. Error bars on each bar plot represent the standard deviation for the relevant phenotype, \* and \*\* represent the significant difference between conventional bread wheat (CBW) and synthetic bread wheat (SBW) at  $P < 0.05$  and  $0.01$ , respectively for student's t-test, **ns** represent non-significance difference between both CBW and SBW for the relevant phenotype

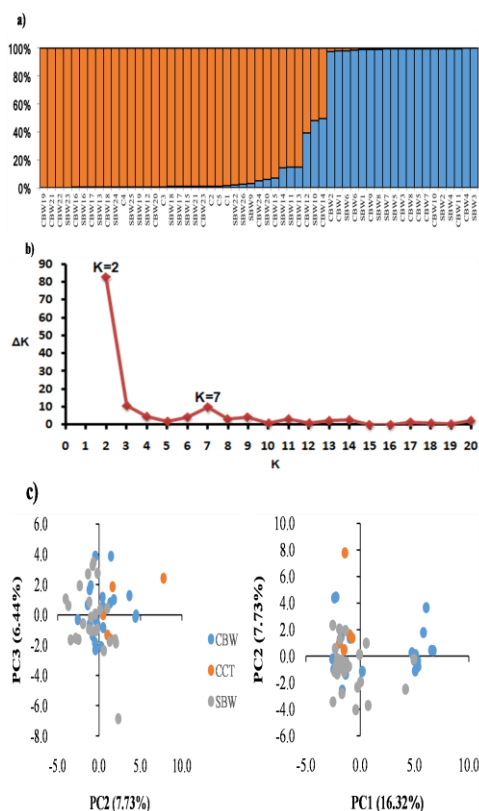
observed for *Vrn-A1* and *Vrn-D1* genes, except that GY and heading time both differed significantly. At *Rht* loci, all the SBW with wild-type *Rht-B1* and *Rht-D1* alleles were superior for GY as compared to CBW (Fig. 1).

## Discussion

There is significant genetic bottleneck arise from the limited hybridization events between durum wheat and *Ae. Tauschii* during evolution of bread wheats (Faheem et al., 2015; Rasheed et al., 2018). Therefore, synthetic hexaploid wheat

is seen as a way to increase genetic diversity in bread wheat (Ogbonnaya et al., 2013). The SBW lines included in this study clearly showed superior performance under terminal drought stress conditions over CBW. These findings are consistent with those reported previously under water-stressed field conditions (Lopes and Reynolds, 2011). Similar findings have been reported by Reynolds et al. (2007) where two water regimes i.e., full irrigated and post-anthesis drought stress were used to assess 2 SBW and their recurrent parents. There was 24% increase in yield in synthetic derivatives, 57% increase in biomass and 41%





**Fig. 2:** Population structure analysis; (a) Structure based on  $K=2$ ; (b) Likelihood of the appropriate  $K$  based on  $\Delta k$ ; (c) Principal component analysis based on SSR markers

higher water-use efficiency in SBW than their recurrent parents. Lopes and Reynolds (2011) have also reported same findings in same water conditions (drought and control) and found that in drought, there was 26% increase in yield in synthetic derived wheats as compared to their recurrent parents. Similarly, yield advantage of up to 8% over Australian conventional bread wheat cultivars was also reported (Rathey and Shorter, 2010). In our case, 8% yield advantage was observed which could be attributed to improved plant architecture and partially due to the confounding effects of flowering time.

The plants escape plan from the stress by completing developmental ontogeny before the onset of stress (Farooq *et al.*, 2014; Kazan and Lyons, 2016), although it may be associated with reduced potential for biomass production (Blum, 2009). However, in this case, the effect was relatively marginal with SBW lines showing just a day or so fewer days to maturity (Fig. 1). The confounding effects of flowering time may be attributed to the yield performance of SBW and likely to be contributed by *Ppd-D1* and *Vrn-D1*. Although we only identified the photoperiod insensitive type (*Ppd-D1a*) and sensitive type (*Ppd-D1b*) alleles base on 2kb indel, however it has been shown that there are four more polymorphisms within *Ppd-D1* (Guo *et al.*, 2010). Especially in a case in which wild accessions of *Ae. tauschii* are used as ancestor of germplasm like SBW in this study.

The superior performance of SBW over CBW having *Ppd-D1* can be attributed to these novel polymorphisms. Similar trend for *Vrn-D1* was observed which indicated the presence of novel unidentified polymorphism in SBW. There was a positive and highly significant correlation between PM and DF ( $r=0.67$ ), however negative correlation was seen among all other traits. Early flowering leading to earliness in physiological maturity can be a reason adaptability to drought stress. These factors clearly gave yield advantage to escape drought. Earliness is the primary stage for breeding in terminal drought condition (Blum, 2009) and in this study earliness was seen in almost all genotypes in drought contrary to control. As drought was given at pre-anthesis stage (most sensitive period of grain setting) which somewhat permits drought escape in control condition.

The strong correlation of PH with yield components indicated that genotypes with more height under drought stress after anthesis can lead to more yields in terms of grains per spike and other yield related parameters. This has been well validated in bread wheat that varieties with wild-type *Rht-B1* and *Rht-D1* genes produce long coleoptile which ultimately help in more seedling emergence and water uptake under water deficit conditions (Trethowan *et al.*, 2001; Rebetzke *et al.*, 2007). In our experiment, SBW with wild-type *Rht-B1a/Rht-D1a* gene out-yielded the CBW and varieties with *Rht-B1b* in terminal drought stress

conditions. There was weak but positive correlation was present between PH and TGW in control ( $r=0.29$ ). Whereas, a strong positive correlation ( $r=0.52$ ) was present between these two in drought. This was in accordance to the findings of van Ginkel *et al.* (1998). A strong association was also seen between SPL and SPP in both drought ( $p \leq 0.01$ ) and control ( $p \leq 0.05$ ) conditions. In accordance to the findings of Zhang *et al.* (2010), there was a significant correlation of these with GS ( $r=0.29$ ) in drought condition only. It can be deduced that in terminal drought conditions, wheat genotypes couldn't grow kernels from all kernel spots. It mostly occurs at pre-anthesis and post-anthesis phases of growth and eventually limiting grain yield.

In drought stress condition, PH, GS, SPL, and SPP had highest coefficients of correlation to grain yield i.e.,  $r=0.53$ ,  $0.62$ ,  $0.59$ , and  $0.80$ , respectively (Izanloo *et al.*, 2008). Blum and Pnuel (1990) revealed that maintenance in GS gained a lot of importance in drought stress conditions. It can be suggested that higher grain yield can be achieved by increasing TGW along with increasing number of grains per unit area. However, it is very challenging due to the negative or poor association between grains per unit area and TGW. One of the reasons are decreased availability of nutrients and assimilates to single grain. As number of grains per unit area increases, the resources to grain-filling also reduces and limited sink is seen in wheat in ideal conditions (Borghi *et al.*, 1986). It has been proposed that SBW can be used an alternate tool to integrate exotic and vital alleles to increase grain yields (Ogbonnaya *et al.*, 2007). Another observation was that there exists a negative yet significant correlation ( $r = -0.27$ ) between GY and DF. It can be interpreted that genotypes with early flowering showed higher yield in drought stress condition. From this study, it can be proposed that results for correlation can provide necessary results to select best genotypes for GY in drought.

Based on the SSR markers, two sub-populations,  $K=2$  were distinct on the basis of highest  $\Delta K$  value (Fig. 2a and 2b). The first group comprised of 20 accessions that are mostly CBW, while second groups comprised of 35 accessions with higher proportion of SBW. You *et al.* (2004) had the same findings as discussed here in this study. Similar characteristics in genome wide association mapping and in population structure have also been used by Neumann *et al.* (2011). In that study, DArT markers in wheat were used and accessions were allocated to one of the two subpopulations ( $\Delta K$ ) at  $K=2$ . Recently, McIntyre *et al.* (2014) observed the preferential retention chromosome region in synthetic derivatives with potential novel alleles for economic traits. This justifies the exploitation of synthetic hexaploids for wheat genetic improvement especially for stress resistance. It can be conferred from current findings that *Ae. tauschii* is the major source to introgress promising alleles for not only of drought tolerance but also of many yield and yield components such as GY, SPP, TGW, and GS. This QTL is derived from

SHW (Syn 84) (parent in backcross) and possess exotic allele (s). Identification of these potential alleles in SBW for increased GS and TGW on D-genome chromosomes and utilizing them in wheat breeding program has been suggested in many studies (McIntyre *et al.*, 2014; Rasheed *et al.*, 2014). Similarly, some unique alleles were also observed, for example *Xgwm304-5AL<sub>116bp</sub>* is unique allele observed only in SBW10, which has good agronomic features under drought stress. These findings support the superior agronomic performance of SBW over CBW and suggest that unique alleles retained within SBW make them more adaptable in terminal drought stress.

## Conclusion

Current work provided detailed knowledge about the agronomic superiority of synthetic wheat derivatives over the conventional wheat cultivars in context of major adaptability genes. We showed that synthetic wheats are one of the promising resources for many vital characteristics including SPP, GY, PM, PH, TGW, DF, GS, and SPL. Our results would help wheat breeders to exploit this diversity carrying more beneficial alleles and also narrowed down four genotypes (SBW8, SBW10, SBW13 and SBW24) for recombination breeding.

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