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Full Length Article

Population Structure, Genetic Diversity and Selection of Terminal Heat Stress Tolerant Synthetic Derivative Wheat Genotypes

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Abstract

Due to climate change, terminal heat stress has become a major constraint to wheat production throughout the world. Wild relatives of wheat have proved their potential to meet these challenges. In the following study, we explored 52 synthetic derivative wheats (developed through crossing of wild parents) for genetic diversity, population structure and selection of heat tolerant genotypes. Under field conditions, plant material was evaluated under terminal heat stress for two years *i.e.*, 2016–18. Heat tolerance was assessed through the plant traits like days to heading (DTH), days to physiological maturity (DPM), canopy temperature (CT), SPAD value (SPD), flag leaf length (FL), spikes per plant (Sp./P), plant height (PH), spike length (Sp.L), individual spike weight (ISW), harvest index (HI), thousand grain weight (TGW), grains per spike (G/Sp) and grain yield (GY). Analysis of variance depicted significant variation among genotypes, treatments and years for the studied traits. Heat tolerant genotypes *i.e.*, BW/SH-126, BW/SH-17, BW/SH-116, BW/SH-42, BW/SH-30, BW/SH-138, BW/SH-113, BW/SH-73, BW/SH-79 and BW/SH-146 were identified by Biplot analysis based on heat stress tolerance index. Molecular analyses, following the characterization of genotypes with SSR markers, revealed average PIC and gene diversity values as 0.27 and 0.332, respectively. Population Structure analysis divided the genotypes into three sub-populations. The analysis of molecular variance revealed a maximum of 79% variation within the populations and 21% among populations. The results revealed that studied germplasm has a great potential to overcome the losses due to terminal heat stress which occur due to late sowing and early arrival of spring and summer in climate change scenario.

Keywords: Terminal heat stress; Genetic diversity; Climate change; Population structure analysis

Introduction

Wheat (*Triticum aestivum* L.) is an important and major cereal crop for human nutrition in global food chain (Bonjean and Angus 2011). It is 3rd leading cereal crop after maize and rice that together produce nearly 57% world's calories from agricultural sources (Tilman *et al.* 2011). Since past 60 years, the improvement in the classical breeding procedures has made wheat crop as an important cereal with production of 749 million tons after maize with 1.03 billion tons production (FAO 2016). Due to climate change across the globe, heat stress is among major hazards to wheat production (Hall 2001; Farooq *et al.* 2011). Prolonged exposure to temperature with ranges from 20–30°C and short periods of heat shock with temperature above 32°C may result in damage to plant growth and development.

Transient temperature may cause yield reductions up to 10–15% (Wardlaw *et al.* 1989; Peet and Willits 1998). Globally, rise in mean temperature per decade is about 0.3°C (Jones *et al.* 1999) which may reach up to 1°C by the years 2025 and 3°C by 2100. Temperature rise may alter agricultural crops growing season by sowing them earlier than their actual time (Porter 2005). Spring wheat is greatly affected by high temperature stress at anthesis and grain filling stages which resulted in 7% decrease in kernel yield (Guilioni *et al.* 2003). Similarly, Giaveno and Ferrero (2003) also reported sensitivity of number of grains and weight to high temperature stress in wheat.

Due to challenges being posed by high temperature stress, development of heat tolerant crops is need of the time for sustainable food production. For this purpose, a combination of conventional and molecular breeding methods is being used. For the development of heat tolerant wheats, selection of distant parents with desirable traits is a prerequisite. Previously, selection of distant parents was

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made based on morphological screening but now a day with the advent of molecular markers, selection coupled both conventional and molecular markers is more successful (Ahmad *et al.* 2014).

Several molecular markers are being used to identify genetic diversity and selection of genotypes such as SSRs, SNPs, DArT, RFLP, and AFLPs. Among these markers systems for genotyping, SSRs are the frequently used molecular markers (Ahmad *et al.* 2014). Several studies have shown the detection of polymorphisms in wheat accessions using microsatellite SSR markers (Khan *et al.* 2015). Population structure and genetic diversity in various crops have been assessed using SSR markers (López-Gartner *et al.* 2009; Blair *et al.* 2010). For population genetics analysis, SSR markers are widely used in comparison with SNP markers (Van Inghelandt *et al.* 2010).

The population structure analysis has been conducted by many researchers for different self-pollinating cereal crops to investigate germplasm genetic diversity (Huang *et al.* 2002**)**. Presence of distinct groups in the germplasm occurs due to selection under various geographic origin, mating habit, selection driven by human, environmental factors and genetic drift (Buckler and Thornsberry 2002). Previous studies conducted for determination of genetic diversity and population structure showed high values of polymorphism information content due to presence of mutational properties in the population (Dreisigacker *et al.* 2005; Reif *et al.* 2005; Roussel *et al.* 2005).

In the present study, we evaluated 52 synthetic derivative wheat genotypes under terminal heat stress imposed by late sowing. We identified the terminal heat tolerant genotypes by performing principal component biplot analysis using data set of relative performance C-S/C for normal and stress conditions. Population structure, neighbor joining tree analysis and analysis of molecular variance were conducted to explore the genetic diversity among the genotypes for terminal heat stress tolerance.

Materials and Methods

Plant materials and phenotypic characterization

Fifty-two D genome synthetic derivative wheat genotypes were evaluated at experimental area of the department of Plant Breeding and Genetics, Bahauddin Zakariya University, Multan, Pakistan during indigenous wheat cropping seasons of 2016–2017 and 2017–2018 (Table 1). Experiment was conducted under normal and high temperature stress conditions. Terminal heat stress or high temperature stress at grain filling stage was created by delaying planting in heat stress trial. Fifty-two genotypes were sown on Nov-15, during both years and considered as normal trail whereas; to expose same genotypes to terminal heat stress one trial was sown on Dec 30, during both years and hereafter referred as heat stress trial. Experiment was performed in randomized complete block design (RCBD) using three replications. Each genotype was sown on a row of 5 m length. Plant to plant and row to row distance was kept at 12 cm and 30 cm, respectively. Sowing was done with handheld drill. Distance between plants was maintained by manual thinning after first irrigation at proper moisture conditions. Grain filling stage of early sown genotypes was in March where temperature was low while foe late sown it was in April where temperature was higher in both years (Fig. 1). Recommended indigenous agronomic practices were followed for conducting research trial.

Thirteen agro-morphological and physiological traits *viz*., days to heading (DTH), days to physiological maturity (DPM), canopy temperature (CT), SPAD value (SPD), flag leaf length (FL, cm), spikes per plant (Sp./P), plant height (PH, cm), spike length (Sp. L, cm), individual spike weight (ISW, g), harvest index (HI), thousand grain weight (TGW,

Table 1: Lists of genotypes used in this study

S. No.	Names	S. No.	Names
G1	BW/SH-5	G27	BW/SH-178
G2	BW/SH-126	G28	BW/SH-122
G3	BW/SH-161	G29	BW/SH-49
G4	BW/SH184	G30	BW/SH-170
G5	BW/SH-156	G31	BW/SH-78
G6	BW/SH-158	G32	BW/SH-113
G7	BW/SH-181	G33	BW/SH-47
G8	BW/SH-17	G34	BW/SH-114
G9	BW/SH-22	G35	BW/SH-71
G10	BW/SH-181A	G36	BW/SH-73
G11	BW/SH-94	G37	BW/SH-176
G12	BW/SH-207	G38	BW/SH-79
G13	BW/SH-169	G39	BW/SH-20
G14	BW/SH-116	G40	BW/SH-106
G15	BW/SH-15	G41	BW/SH-198
G16	BW/SH-171	G42	BW/SH-204
G17	BW/SH-104	G43	BW/SH-173
G18	BW/SH-42	G44	BW/SH-1
G19	BW/SH-31	G45	BW/SH-35
G20	BW/SH-44	G46	BW/SH-154
G21	BW/SH-30	G47	BW/SH-144
G22	BW/SH-48	G48	BW/SH-209
G23	BW/SH-174	G49	BW/SH-146
G24	BW/SH-138	G50	BW/SH-175
G25	BW/SH/190	G51	BW/SH-52
G26	BW/SH-28	G52	BW/SH-6

Table 2: List of Thirty SSR Primers used in this study

S. No.	Name of SSR	S. No.	Name of SSR
1	WMC-70	16	WMC-557
2	WMC-75	17	WMC-570
3	WMC-96	18	WMC-581
4	WMC-276	19	WMC-598
5	WMC-289	20	WMC-602
6	WMC-332	21	WMC-604
7	WMC-382	22	WMC-640
8	WMC-407	23	WMC-661
9	WMC-434	24	WMC-667
10	WMC-474	25	WMC-684
11	WMC-477	26	WMC-734
12	WMC-479	27	WMC-764
13	WMC-500	28	WMC-770
14	WMC-508	29	WMC-786
15	WMC-526	30	WMC-807

g), grains per spike (G/Sp.) and grain yield (GY, g) were recorded. Data were recorded from five randomly selected plants from each genotype from all three replications.

Statistical analysis

Analysis of variance was performed by using Minitab software. To assess genetic diversity among genotypes, principal component analysis (PCA) was performed using XLstat. For PCA, a data set [(C-S)/C)] × 100 based on genotypic relative performance under normal and heat stress conditions was performed as suggested by (Ivandic *et al.* 2000). Biplot analysis was performed based on PCA results which helped in identification of stress tolerant genotypes.

Genotypic Characterization

DNA extraction and SSR analysis: High quality DNA was extracted using ten days old plant leaves by CTAB method as suggested by Khan *et al*. (2004). DNA quantification was done using Nano photometer *IMPLEN.* The samples were diluted to approximately 30 ng/μL. A total of 30 SSR markers were screened for 52 wheat genotypes, out of which 13 polymorphic SSRs were selected for final analysis (Table 2). Thermal profiles of these SSR primers were obtained from GrainGenes data base (http://wheat.pw.usda.gov). PCR was performed in 20 μL reaction. PCR reaction mixture conditions were as 1 μL (30 ng/μL) DNA, 2 μL (10x PCR buffer with 500 m*M* NH_2SO_4), 2.5 μL (25 m*M*) $MgCl_2$, 0.5 μL (10 m*M*) dNTPs, 0.5 μL (20 m*M*) of every forward and reverse primers, 1 μL Taq Polymerase and 12.8 μL distilled water. Products of PCR were separated on 2% high resolution agarose gel.

Genetic diversity analysis: A binary data matrix constructed based on presence (1) and absence of bands (0) which was further used for genetic diversity analysis. The parameters *viz*., number of alleles per locus, major allele frequency, gene diversity, heterozygosity and polymorphism information content were estimated using *Power Marker* v3.25 (Liu 2005). *DARwin* v.6.0.13 was used to perform neighborhood joining tree analysis with 1000 bootstrap value (Perrier and Jacquemoud-Collet 2006). GenALEx 6.5 was used for analysis of molecular variance within and between sub-populations identified by structure analysis (Peakall and Smouse 2012).

Population structure analysis: For determination of population structure (sub-populations= K) among genotypes, a model-based clustering analysis using *STRUCTURE* v2.3.4 was performed (Pritchard *et al.* 2000). By applying an admixture and correlated allele frequency model, the parameter set was adjusted for each run with 30,000 burn-in period and 30,000 MCMC repeats. The number of sub-populations (K) ranged from 1 to 20. For each K, 10 runs were performed separately. The best K value was obtained using LnP(D) value ranging from 1 to 20 subpopulations. The value of LnP(D) peaked in the graph was used as K and again 10 runs were performed with 100,000 burn-in period and 100,000 MCMC repetitions.

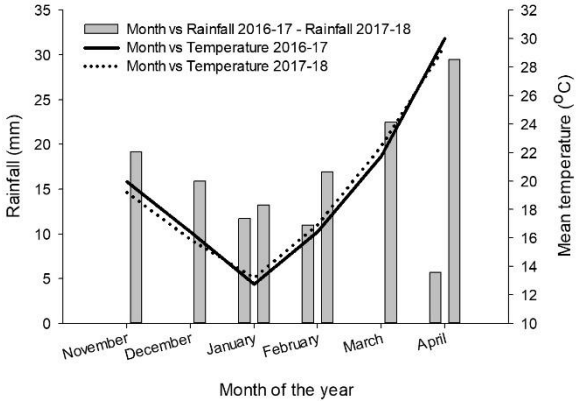

Fig. 1: Weather data of experimental site for the experimental duration of two years (2016-2018)

Results

Analysis of variance depicted a significant ($P \leq 0.05$) variation among genotypes and treatment for all studied traits. The interaction of genotype and treatment (G×T) also observed significant ($P \leq 0.05$) for all traits in exception to individual spike weight (Table 3).

Under normal conditions, genotypes mean performance showed that DTH ranged from 84 to 93 days and from 92 to 107 days during both years, respectively whereas under stress condition reduction in DTH was observed which ranged from 66 to 87 days and from 71 to 95 days respectively. Similarly, for DPM under normal condition, value for the genotypes ranged from 127 to 134 and from 115 to 131 in first and sec year respectively whereas, under heat stress it ranged from 104 to 129 and from 91 to 119 days in first and sec year respectively. For grain yield mean under normal condition the genotypes were ranged from 30.2 to 66.4 g and from 24.8 to 54.9 in first and sec year respectively whereas under heat stress it ranged from 19.5 to 48.3 and 11.2 to 38.9 in first and sec year respectively (Table 5).

Broad sense heritability (H^2B.S.) analysis was performed to determine the proportion of variation which can be transferred to next generation. Moderate to low broad sense heritability values were found for all the traits studied. Heritability values ranged from 0.30 to 0.70%. PH showed highest heritability whereas lowest value was depicted by Sp/P (Table 5).

Principal component analysis (PCA): Pattern of variation among the genotypes was determined using principal component analysis. As ANOVA depicted significant differences for years for all the traits therefore, a separate PCA was performed for the years 2016–17 and 2017–18 (Table 4). Highest proportion of variation was recorded by the first five PCs (62.8%) during year 2016–17. The amount

Table 3: Analysis of variance (ANOVA) for 13 traits under normal and stress condition for years 2016-17 and 2017-18

SOV	DF	DTH	DPM	CT	SPV	FLL	Sp./P	PH	Sp. L	ISW	HI	TGW	G/Sp	GY
G	51	40.7*	19.6*	4.155*	154.16*	57.09*	17.55*	385.1*	7.846*	1.127	0.0211*	108.4*	418.1*	238.9*
Y	1	9572.3	10469.8	884.384	2745.96	324.09	996.37	12520.2	29.263	269.465	2.68869	12174.7	303.1	14638.4
T	1	56354	68628.1	0.249	6509.35	1721.69	1775.25	13829.3	702.166	287.728	1.84024	2740.4	20370.6	33100.1
REP	2	332.1	299.9	8.458	9.35	4.74	5.57	132	4.399	2.85	0.04469	184.2	9.1	165.2
G*T	51	24.9	14.3	3.33	35.46	29.5	13.43	43.2	2.278	0.735	0.01351	44.8	76.9	60.5
Error	208	7.5	4.5	2.037	14.62	6.75	5.95	11.9	1.596	0.691	0.00593	12.7	17.6	11.9

DTH= Days to heading, **DPM**= Days to physiological maturity, **CT**= Canopy temperature, **SPV**= SPAD value, **FLL**= Flag leaf length, **Sp./P**= Spike per plant, **PH**= Plant height, **Sp. L**= Spike length, **ISW**= Individual spike weight, **HI**= Harvest index, **TGW**= Thousand grain weight, **G/Sp.**= Grains per spike, **GY**= Grain yield

Table 4: The descriptive statistics of 13 traits for 52 genotypes under control and stress conditions for years 2016-17 and 2017-18

	2017				2018				
	Control				Stress				
Variables	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range	h^2
DTH	90.1 ± 0.3	84-93	74.2 ± 0.55	66-87	99.1 ± 0.4	92-107	77.7 ± 0.4	71-95	0.4714
DPM	129.5 ± 0.2	127-134	109.7 ± 0.45	104-129	120.5 ± 0.3	115-131	99.7 ± 0.5	91-119	0.5631
CT	24.6 ± 0.07	23.2-25.8	23.3 ± 0.17	21.3-25.9	26.4 ± 0.1	24.1-29.9	25.2 ± 0.1	22.9-29.9	0.3962
SPV	51.8 ± 0.7	40.3-64.3	46.7 ± 0.9	23.4-62.4	49.6 ± 0.4	42.4-57.4	38.8 ± 0.5	30.5-50.7	0.5979
FLL	24.1 ± 0.5	15.5-33	20.3 ± 0.5	14-28	23.8 ± 0.4	18-31.2	18.8 ± 0.5	6.2-27.8	0.6110
Sp./P	8 ± 0.3	3-13	5.74 ± 0.3	2-12	12.2 ± 0.4	6.8-19.2	6.8 ± 0.1	4.2-11.6	0.3060
PH	94.6 ± 1	78.4-122.7	87 ± 1.2	69.7-120.5	87.5 ± 0.9	72.9-102.2	75.4 ± 0.9	60.7-92	0.6992
Sp.L	12.4 ± 0.2	10-16.3	10.6 ± 0.19	7.9-14	12.3 ± 0.2	8.5-15.2	9.4 ± 0.1	6.3-12.8	0.4125
ISW	4.5 ± 0.1	2.56-5.9	3.14 ± 0.09	2.3-5.9	2.6 ± 0.05	2.02-3.5	1.8 ± 0.04	1.22-2.6	0.4984
HI	0.73 ± 0.01	0.51-0.87	0.65 ± 0.01	0.45-0.8	0.7 ± 0.03	0.67-0.82	0.6 ± 0.05	0.5-0.8	0.6151
TGW	42.5 ± 0.5	32-51	38.4 ± 0.6	25.7-46.3	32.3 ± 0.5	22.5-42.6	26.4 ± 0.6	18.2-36.8	0.6758
G/Sp	52.2 ± 1	36-78	44.4 ± 0.9	27.6-61.5	53 ± 1.2	32.8-76.3	39.8 ± 1.2	20.6-68.3	0.6767
GY	48.7 ± 1.1	30.2-66.4	33 ± 0.7	19.5-48.3	37.4 ± 0.9	24.8-54.9	23.8 ± 0.8	11.2-38.9	0.6151

Table 5: PCA for all traits under normal and heat stress condition based on (C-S/C) data set for the years 2016-17 and 2017-18

Year	2017		2018	
Variable	PC1	PC2	PC1	PC2
DTH	0.550	0.145	-0.258	0.051
DPM	0.461	0.001	-0.168	0.041
CT	-0.111	-0.126	-0.006	0.063
SPV	-0.193	0.547	-0.214	-0.559
FLL	-0.342	-0.315	-0.083	-0.317
Sp./P	0.301	0.107	0.076	0.155
PH	-0.068	-0.289	0.211	0.572
Sp. L	-0.122	-0.159	0.110	-0.401
ISW	0.056	0.252	0.503	-0.116
HI	0.118	-0.105	0.491	-0.122
TGW	-0.362	0.523	0.128	-0.117
G/Sp.	0.076	-0.258	0.167	-0.019
GY	-0.231	-0.175	0.502	-0.155
Eigen value	2.326	1.680	3.391	1.869
Variability%	17.890	12.920	26.081	14.375
Cumulative%	17.890	30.810	26.081	40.456

of variation explained by first PC during 2016–17 was 17.8% variation with major contribution by DTH, DPM, Sp./P, and FLL. The sec PC showed major diversity for SPD and TGW. Maximum variation accounted by 2nd PC was 12.9%. During year 2017–18 the maximum variation accounted by first five PCs was 71.68%.

The maximum variation with positive Eigen vectors for yield related traits was observed in first PC of year 2017–18 as compared to year 2016–17. First PC for year 2016–17 was more related to plant physiological traits such DPM and DTH. Sec year first PC accounted with 26% of total variation which is higher than first year's first PC which showed 17.8% variation (Table 4). The first PC of 2017–18 accounted maximum variation for ISW, HI and GY whereas; the sec PC contained maximum variation for PH, SPD and SL. PCA for both years conducted on the basis of relative performance of normal and stress condition using C-S/C data set explained 62.8% variation in the year 2016–17 while in the sec cropping season of 2017–18 it showed 71.8% variation for first five PCs (Table 4).

Association among traits and recognition of stress tolerant genotypes were determined using biplot analysis. The trait vectors demonstrated the presence of greater and lesser diversity by each trait due to occurrence of long and short length of polygons on biplot chart (Fig. 2 and 3). The first cropping season 2016–17 explained maximum

variation for DTH, DPM, SPD and TGW. The genotypes with major diversity having negative Eigen values were G-2, G-24, G-36 and G-37 during year 2016–17. Whereas in sec cropping season 2017–18 the traits accounted for major diversity were DTH, DPM, PH, ISW, HI and GY. The genotypes accounted for major diversity were G-4, G-14, G-16, G-31, G-36 and G-40. The ISW, HI and GY showed close angle to each other, it could be concluded that these traits were responsible for yield production. The genotypes with longer vector lengths and greater diversity accounted in PCA were considered as tolerant genotypes (BW/SH-126, BW/SH-17, BW/SH-116, BW/SH-30, BW/SH-42, BW/SH-138, BW/SH-113, BW/SH-73, BW/SH-79 and BW/SH-146).

Genetic diversity analysis: In the present study, 52 synthetic wheat genotypes were analyzed with 30 SSR markers. On the basis of polymorphism, 13 SSR markers were selected for further analysis. SSR markers and their parameters of genetic diversity are shown in (Table 6). A total of 27 alleles were amplified using 13 SSR markers with 2.07 average alleles per locus. The maximum number of alleles (3) was detected by WMC661. The average PIC value obtained from 13 markers was 0.2709 with minimum value of 0.0848 by WMC602 and maximum value of 0.4846 by WMC661. All the markers exhibited zero value for observed heterozygosity (Ho), while the expected heterozygosity or gene diversity (He) exhibited maximum gene diversity of 0.5473 (WMC661) followed by 0.4998 (WMC332) and minimum value of 0.0887 by WMC602 with an average of 0.3320. The average value of major allele frequency of these polymorphic markers was 0.7542 with maximum and minimum value of 0.9767 by WMC786 and 0.5102 by WMC332, respectively (Table 6).

Neighbour joining tree analysis: The un-rooted tree was constructed with unweighted neighbor joining tree method for the cluster analysis of 13 SSR markers which separated the 52 genotypes into three major and seven minor clusters (Fig. 4). The genotypes present in the Cluster I exhibited 4 moderate and 2 susceptible genotypes against stress. Cluster II consisted of 20 genotypes with 4 highly tolerant, 12 moderately tolerant and 4 susceptible genotypes. The genotypes (BW/SH-116, BW/SH-30, BW/SH-73 and 176) were highly tolerant, while the genotypes (BW/SH-28, 173, BW/SH-47 and BW/SH-156) were found susceptible. Cluster III sub divided into 4 minor clusters contained many tolerant genotypes with greater diversity and high yielding performance. It contained 6 highly tolerant genotypes, 17 moderately tolerant and 3 susceptible genotypes. Hence, Cluster III considered as highly tolerant cluster with maximum tolerant genotypes.

Population structure analysis: The Bayesian based clustering approach was used in *STRUCTURE* software to estimate number of sub-populations present among studied populations. The LnP(D) graph showed first highest peak at K=3 among twenty sub-populations. Hence, 52 genotypes were divided into three sub-populations. Number of

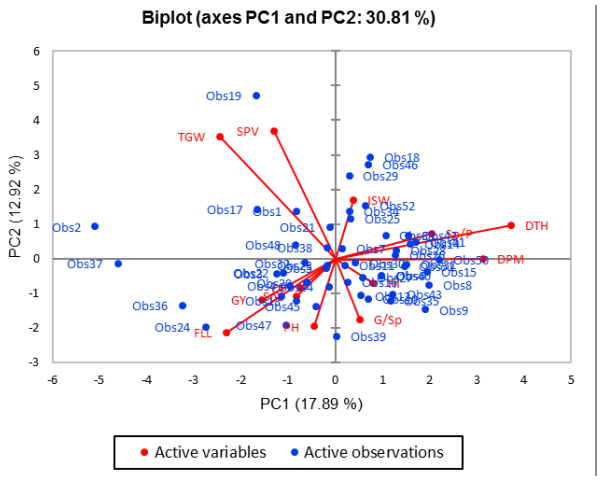

Fig. 2: Biplot of genotypes based on C-S/C data set for year 2016-17

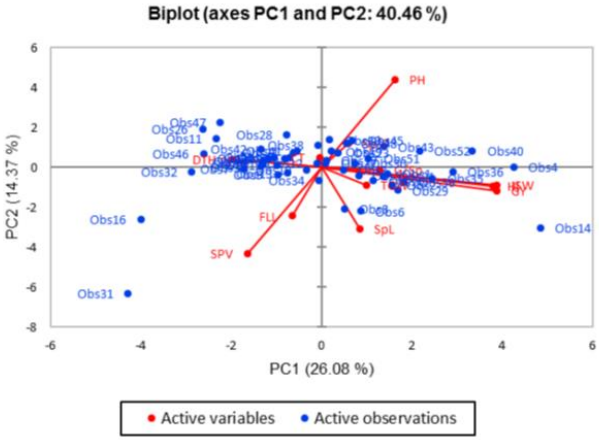

Fig. 3: Biplot of genotypes based on C-S/C data set for year 2017-18

genotypes including in various sub-populations were 12, 20 and 20, respectively (Fig. 5). The sub-population I contained 12 genotypes accommodating weakly tolerant to susceptible genotypes under heat stress condition. In sec sub-population, genotypes were moderately tolerant to ambient temperature with few susceptible genotypes. The third sub-population possessed highly tolerant genotypes under both years. The analysis of molecular variance explained that major variation present within sub-populations is shown in the (Table 8). The PhiPT value 0.207 within sub-populations was significant ($P < 0.007$), representing that the data was significant and the sub-populations differentiation among 52 genotypes were significant.

The genotypes are divided into three sub-populations and the genetic variability observed for these three sub-populations is shown in (Table 7). The average gene diversities for each SSR locus in Populations (1, 2 and 3) of synthetic wheats were found 0.257, 0.202 and 0.24 respectively. For each sub-population, mean PIC values were 0.207, 0.164 and 0.192 respectively (Table 5). Comparative study revealed that Population 1 genotypes

Table 6: Genetic diversity parameters of 13 SSR markers used for genotyping 52 synthetic genotypes

Marker	Major Allele Frequency	Allele No	Gene Diversity	Heterozygosity	PIC
WMC70	0.7419	2.0000	0.3829	0.0000	0.3096
WMC75	0.6923	2.0000	0.4260	0.0000	0.3353
WMC332	0.5102	2.0000	0.4998	0.0000	0.3749
WMC382	0.7000	2.0000	0.4200	0.0000	0.3318
WMC474	0.9038	2.0000	0.1738	0.0000	0.1587
WMC479	0.5909	2.0000	0.4835	0.0000	0.3666
WMC553	0.8235	2.0000	0.2907	0.0000	0.2484
WMC581	0.5745	2.0000	0.4889	0.0000	0.3694
WMC602	0.9535	2.0000	0.0887	0.0000	0.0848
WMC640	0.8846	2.0000	0.2041	0.0000	0.1833
WMC661	0.6098	3.0000	0.5473	0.0000	0.4846
WMC786	0.9767	2.0000	0.0454	0.0000	0.0444
WMC807	0.8431	2.0000	0.2645	0.0000	0.2295
Mean	0.7542	2.0769	0.3320	0.0000	0.2709

Table 7: Diversity information in all three sub-populations

Sub-Population	No. of Genotypes	Major Allele Frequency	Allele No.	Gene Diversity	Heterozygosity	PIC
Population 1	12	0.7790	1.6923	0.2579	0.000	0.207
Population 2	20	0.8553	1.6153	0.2029	0.000	0.164
Population 3	20	0.8144	1.6923	0.2409	0.000	0.192
Mean	17.3333	0.8162	1.6666	0.2339	0.000	0.188

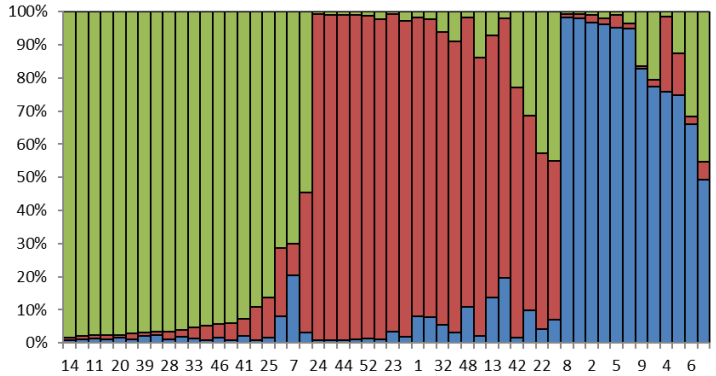

Fig. 4: Population structure analysis revealed through SSR markers for 52 genotypes

(GD=0.257, PIC=0.207) possessed higher genetic variability than rest of sub-populations. However, average number of alleles in Population 1 and Population 3 were similar (1.692) but higher than Population 2 (1.615).

Discussion

Rise in temperature is a serious threat to global crop production (Hall 2001) which may cause alteration in the growing season of crops due to the onset of high temperature at crop maturity stage (Porter 2005). A prominent variation in yield parameters occurred due to temperature driven mechanisms. The high temperature (34˚C) at grain filling stage leads the crop to senescence stage and responsible for reduction in grain yield (Fischer 1980; Rane and Nagarajan 2004; Zhao *et al.* 2007). Exposure to high temperature stress results in altered expression of genes at molecular level (Iba 2002). Therefore, to combat the menace of high temperature stress, genetic resources should be explored to identify heat tolerant material.

Genetic diversity is a pre-requisite to identification of stress tolerant germplasm lines. *Ae. tauschii* a wheat ancestor, is a good source of many genes for stress resistance. It contains significant genetic diversity which could be exploited against biotic and abiotic stresses (Ogbonnaya *et al.* 2013; Sehgal *et al.* 2015). Wheat breeders have developed various hexaploid wheat synthetics by crossing *Ae. tauschii* (diploid) and tetraploid wheat. Keeping in view the importance of synthetic germplasm, the 52 synthetic derivative wheat genotypes were used in this study to determine genetic diversity and selection of heat tolerant wheats. Genotypes were experimented in two different environmental conditions to find out heat tolerant combinations. Analysis of variance showed promising genetic variability among all genotypes under both conditions. Mean square values of genotypes, treatments and genotype-treatment interaction were highly significant

Table 8: Summary of Analysis of Molecular Variance (AMOVA) for 13 SSR markers among and within sub-populations

SOV	DF	SS	MS	Est. Var.	% TV	*P*
Among populations	2	2.269	1.135	0.055	21%	0.001
Within populations	49	10.25	0.209	0.209	79%	0.001
Total	51	12.519		0.264	100%	0.001

SOV= Source of variation, **DF**= Degree of freedom, **SS**= Sum of squares, **MS**= Mean squares, **Est. Var.**= Estimate variance, %**TV**= Percentage of total variation, ***P***= Probability value

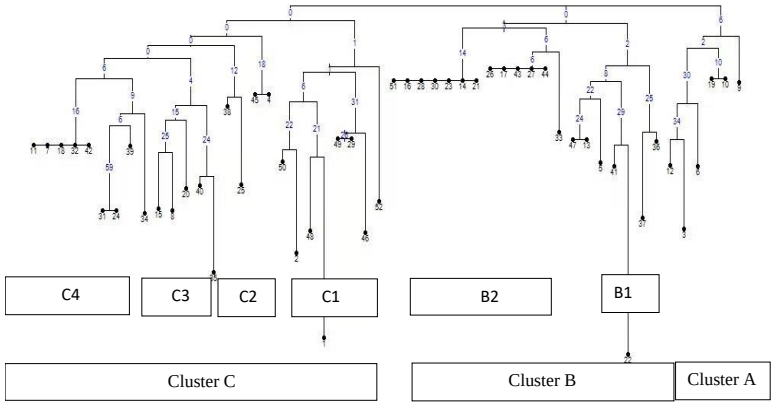

Fig. 5: Unrooted tree using unweighted neighbor joining method showing genetic relation among 52 genotypes based on SSR markers (Number as used for names of the genotypes which correspond to table 1)

for all traits except individual spike weight. Significant ($P \leq 0.05$) variation for genotypes and treatments is an indication that germplasm under study is highly diverse and genotypes have varying level of heat stress tolerance. The amount of difference in the genotypes suggested that there is significant scope to identify heat tolerant genotypes among them.

The descriptive statistics analysis showed high impact of heat stress and significant ($P \leq 0.05$) reduction in all the traits were observed. Medium to low heritability was observed in this study which revealed that environment has major effect for the phenotypic variations (Farshadfar *et al.* 2000; Noorka *et al.* 2012). Our study revealed maximum heritability for plant height (69%) followed by 67% in thousand grain weight and grains per spike. High heritability for yield related traits depicted that both these traits (thousand grain weight and grains per spike can be transferred simultaneously.

Genetic diversity based on phenotypic dataset was explored using principal component analysis (Panthee *et al.* 2006). In this study PCA was conducted using relative performance data set (C-S/C) which revealed genetic diversity for heat tolerance. Previously, Ahmad *et al.* (2014) also used relative performance dataset to identify stress tolerant genotypes in a large panel of hexaploid wheats. Association among different traits can help the breeders to improve various traits for better production (Peterson *et al.* 2005). PCA in sec year showed higher cumulative variation of 71.8% as compared to first year with 62.8% variation. The first two principal components were observed with largest loading values for developmental and yield traits (days to heading, physiological maturity, flag leaf length, spike per plant, SPAD value and thousand grain weight) in first year 2016-17 while in sec year 2017-18 the traits related to yield (plant height, spike length, individual spike weight, harvest index and grain yield) showed higher loading values. Many researchers have reported higher loading values of yield and plant development related traits in first two PCs (Kaya *et al.* 2002; Leilah and Al-Khateeb 2005; Iannucci *et al.* 2011; Khodarahmpour *et al.* 2011; Ahmad *et al.* 2015).

The use of biplot display is a common method to identify stress tolerant genotypes against heat stress and is commonly used for the selection and clustering of genotypes (Talebi *et al.* 2009; Ul-Allah *et al.* 2019). The length of trait vector is an indication of the diversity possessed by the trait (Souri *et al.* 2005; Firincioglu *et al.* 2009; Talebi *et al.* 2009). During 2016-17 the genotypes which appeared as diverse include BW/SH-126, BW/SH-31, BW/SH-42, BW/SH-73, 176, BW/SH-144 whereas; following year the genotypes BW/SH-184, BW/SH-116, BW/SH-171, BW/SH-106 and BW/SH-154 showed major variation.

Thirteen SSR markers produced 27 alleles with 2.07 average alleles per locus for 52 synthetic wheat genotypes. Chen *et al.* (2012) employed 269 SSRs on 90 Chinese winter wheats and found 5.05 average alleles per locus. Roder *et al.* (2002) reported an average of 10.5 number of alleles per locus in 500 European wheat varieties analyzed with microsatellite SSR markers. Yao *et al.* (2009) assessed genetic diversity in 108 wheat genotypes which revealed 5.7 average number of alleles per locus. Similarly, 95 soft winter wheat cultivars produced 4.8 number of alleles per locus (Breseghello and Sorrells 2006).

Genetic variability was assessed in present study using PIC and GD values. In this study, average gene diversity (0.33) and PIC values (0.27) revealed moderate level of genetic variability. This might be due to limited number of markers used for genotyping analysis. Previously, Huang *et al.* (2002) analyzed 998 accessions with 26 SSR loci and observed 0.77 value of gene diversity. Chinese bread wheat germplasm consisting of 250 genotypes were assessed for genetic variability that revealed 0.65 average PIC value (Hao *et al.* 2011). Compared to these reported findings, our results showed less gene diversity and PIC values, which may be due to a smaller number of genotypes and markers used in our study.

Population structure analysis in our study has divided the 52 genotypes into three sub-populations and variance among these populations was calculated by AMOVA. Sub-population 1 consisted of 12 genotypes with major contribution of susceptible genotypes. The 2nd and 3rd sub-populations had 20 genotypes each having tolerant and moderately tolerant genotypes. Many studies have already been conducted using the *STRUCTURE* software for the analysis of sub-populations in germplasms. Yao *et al*. (2009) studied 108 Chinese wheat accessions and identified 9 sub-populations. Four sub clusters were identified among soft winter wheat accessions by (Breseghello and Sorrells 2006). AMOVA showed maximum level of intra-population diversity (79%) and low genetic diversity (21%) among populations. Soriano *et al.* (2016) also found much lower variability between sub-populations (17%) than variation within sub-populations (83%) of durum wheat. The PhiPT value was found significant with P value (0.007), explained that the data used for population structure and AMOVA was significant ($P \leq 0.05$). Wang *et al.* (2017) reported significant variation among three clusters of cassava germplasm with significant ($P \leq 0.05$) PhiPT value and strong gene flow among accessions with Nm 18.73 value. Thus, AMOVA revealed moderate differentiation among groups.

Cluster analysis have shown similarity with the results those obtained from population structure analysis. As the genotypes were divided by population structure into three sub-populations; in the similar way neighbor joining cluster analysis also divided all varieties into three major clusters which were further sub-divided into seven minor clusters. Interestingly, each major group contained of tolerant, moderately tolerant and susceptible genotypes. Therefore, these major groups cannot be ranked as merely tolerant or susceptible. Presence of tolerant genotypes in various groups might be due to different heat tolerant genes or QTLs contained by these genotypes. As heat tolerance is a quantitative trait controlled by various QTLs/genes therefore, different groups among heat tolerant genotypes is quite reasonable. This grouping might be due to limited number of SSR used in this study. Furthermore, most of the synthetic wheat germplasm have been developed using similar parental germplasm stock of diploid and tetraploid species. Therefore, breeder's choice of parents for hybridization and environmental pressure in which the selection of segregating materials carried out results in grouping among genotypes. Previous studies claimed that the subgroups and clusters in large population of cultivars could be formed due to the selection methods and genetic drift (Buckler and Thornsberry 2002; Breseghello and Sorrells 2006).

Conclusion

The study identified synthetic derivative wheat genotypes showing tolerance to terminal heat stress at grain filling stage caused by delayed planting. Based on population structure analysis three sub-populations were identified which were further confirmed by neighbor joining tree method. The study identified following stress tolerant genotypes BW/SH-126, BW/SH-17, BW/SH-116, BW/SH-42, BW/SH-30, BW/SH-138, BW/SH-113, BW/SH-73, BW/SH-79 and BW/SH-146. Use of these genotypes in the breeding program may lead to development of high yielding wheat genotypes tolerant to terminal heat stress caused by either later sowing or climate change.

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