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ASSESSMENT OF GENETIC VARIABILITY FOR HEAT TOLERANCE IN BREAD WHEAT (*Triticum aestivum* L.) USING MORPHO-PHYSIOLOGICAL TRAITS AND MOLECULAR MARKERS

V. Kumar^{1*}, S.A. Desai² and S.S. Biradar²

¹Department of Molecular Biology and Biotechnology, National Institute for Plant Biotechnology (NIPB)- IARI, New Delhi-110012, India. Ph-9459797035.

²University of Agricultural Sciences, Dharwad-580005, Karnataka, India.

*Corresponding Author Email: sharmavinod612@gmail.com

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ABSTRACT

A field experiment was carried out under timely sown (control) and late sown (heat stress) conditions during *rabi* 2017-18 to study the genetic variability, heritability and expected genetic advance for 23 traits in 10 bread wheat genotypes. The genotypes exhibited a wide range of variation for all the traits in both environmental conditions indicating the presence of enough genetic variability in the material under study. The genetic coefficient of variation and phenotypic coefficient of variation were high for grain yield per plot in both control as well as in heat stress conditions. Almost all the traits exhibited high heritability, however, very high values of heritability were observed for leaf waxiness (99%), days to 50 per cent flowering (96.30%) and days to maturity (95.10%) under heat stress conditions. The traits like grain yield, thousand-grain weight and kernel weight per spike showed high heritability (81.26%, 80.30% and 89.00%) and genetic coefficient of variation (30.66%, 10.10% and 24.32%) along with high genetic advance (56.94%, 18.65% and 47.42%). In molecular investigation, eight SSR markers were found to be associated with twelve different morpho-physiological traits. Traits selected based on various parameters could be considered for the development of high-yielding genotypes under heat stress conditions.

Keywords : *Triticum aestivum*, PCV, GCV, Heritability, Genetic advance, Heat tolerance, Polymerase Chain Reaction, SSR Markers.

Introduction

Wheat is the second most popular cereal after rice in terms of output. For 75 percent of India's population, it is the most important food crop. Globally, wheat is grown on about 216 million hectares and produced 760.66 million tons (FAOSTAT; 2023). However, this substantial production confronts difficult challenges, driven not only by the escalating global population projected to exceed 9 billion by 2090 but also by the impacts of climate change (Asseng *et al.* 2018). Climate variability presents formidable barriers to wheat cultivation (Attia *et al.*, 2021 and Yang *et al.*, 2017), with mean surface temperatures anticipated to rise by 1.5 to 6 degrees Celsius or more by the century's end, according to climate change models (Stocker *et al.*,

2013). Given the susceptibility of the starch synthase enzyme to elevated temperatures, particularly exceeding 35°C, there is a notable reduction observed in both grain yield and quality (Mondal *et al.*, 2013 and Kazemi *et al.*, 2021).

Additionally, heat stress restricts the life cycle of a plant, decreases plant height, spike length, chlorophyll content, early senescence and acceleration in the filling, shortening the grain filling duration (GFD) and limiting carbon assimilation which eventually declines grain yield (Riaz *et al.*, 2021, Tariq *et al.*, 2021, and Farooq *et al.* 2011). In light of the foregoing facts and numbers, it is imperative that we discover heat-tolerant wheat genotypes that can be

used in future crop improvement programmes to generate heat-tolerant cultivars.

Evaluating genetic diversity within germplasm based on morphological and biochemical traits can provide valuable insights (Khan and Ali, 2017). Nonetheless, relying solely on these traits presents challenges due to their limited number and susceptibility to environmental influences, as noted by Sharma *et al.* (2014). Alternatively, exploring genetic parameters, particularly through molecular markers, that govern plant heat tolerance offers a promising avenue for the breeding of heat-tolerant wheat varieties, as suggested by Jahan *et al.* (2021) and emphasized by Lu *et al.* (2022). Molecular markers, known as specific fragments of DNA, can be used for the exact estimation of genetic similarity and diversity, because they are independent from the environment, and marker-assisted detection efficiently discovers polymorphism among genotypes (Norouzi *et al.*, 2015). To date, several studies have been performed on heat stress-linked markers in various crop plants such as wheat (Bhusal *et al.*, 2017).

The fundamental job in the genetic improvement of any crop plant is to generate information on the genetic variability and methods of inheritance of the qualities involved.

Materials and Methods

Plant Material and Growth Conditions

To assay the effect of terminal heat stress on wheat yield and physiology under field conditions, diversity analysis was carried out for 100 genotypes (Jaya, 2016), out of which 10 genotypes (Table 1) carrying different features related to heat tolerance were selected for this investigation. Selected genotypes were evaluated in RBD with three replications under two different growth conditions *i.e.* timely sown and late sown, at All India Coordinated Wheat Improvement Project (AICWIP), Main Agricultural Research Station (MARS), University of Agricultural Science (UAS), Dharwad during *rabi* 2017-2018. The genotypes were sown in 2 rows of 3m length with 20 cm × 10 cm spacing. Recommended cultural practices were followed and the plots were protected from weeds, pests and disease by taking up necessary agrochemical applications.

Morpho-physiological parameters

The observations were recorded for DFF- Days to 50 per cent flowering, DM-Days to Maturity, PH- Plant Height (cm), TM-Tillers per meter length, SL- Spike Length (cm), SPS- Spikelets per Spike, GPS- Grains per Spike, TGW-Thousand Grain Weight (g),

GY- Grain Yield (quintals/hectare), KWPS- Kernel weight per spike (g), GFD- Grain filling Duration, SPAD-I, II and III - Chlorophyll content at booting, anthesis and grain filling stage respectively, CT-I, II and III -Canopy Temperature at booting, anthesis and grain filling stage respectively, NDVI-I, II and III - NDVI at booting anthesis and grain filling stage respectively, LW-Leaf Waxiness, MSI-I and II- Membrane Stability Index (%) at anthesis stage and at grain filling stage respectively. The mean of five observations was computed for all the characters except for days to 50 per cent flowering, maturity, and grain yield. The current study evaluated the 10 bread wheat genotypes for genetic coefficient of variation, phenotypic coefficient of variation, heritability, and genetic advance to define and develop acceptable selection indices for yield attributes under heat stress circumstances.

Molecular Investigation

Twenty previously identified molecular markers associated with heat tolerance attributes were employed in this study (Table 2). Genomic DNA extraction from 15-day-old seedlings was performed using the cetyltrimethylammonium bromide (CTAB) method, following the protocol outlined by Sharma *et al.* (2014). The qualitative and quantitative assessment of genomic DNA was done by both spectrophotometric and also by gel electrophoresis at 0.8 per cent agarose gel with known concentrations of uncut DNA. Touchdown polymerase chain reaction (PCR) protocol was used to get the prominent banding pattern in SSR primers (Fig. 1).

Data Analysis

The data obtained were subjected to the biometrical analysis in the WINDOSTAT software package (version?) to find out the analysis of variance, heritability, genotypic coefficient of variation (GCV %), phenotypic coefficient of variation (PCV %), broad-sense heritability (h^2 (bs) %) and genetic advance over a mean (GAM) (Table 4). The estimate of GCV and PCV were classified as low, medium and high (Sivasubramanian and Madhavamenon, 1973). The heritability was categorized as suggested by Robinson *et al.* (1949). Further, the genetic advance was classified by adopting the method of Johnson *et al.* (1955).

Single marker analysis (SMA) was performed to determine the significance and the contribution of the marker towards heat tolerance in the selected wheat genotypes using Win QTL Cartographer version 2.5 (Wang *et al.*, 2007). Analysis of variance (ANOVA) and linear regression (Haley and Knott, 1992) were

used in SMA to check the significance and work out the coefficient of determination (R^2) of each marker towards total phenotypic variation arising from the QTL linked to the marker. The regression results for

each band were confirmed by the student's t-test. The mean performances of genotypes were divided into two groups (according to their banding pattern) and then were examined by t-test.

Table 1: The list of bread wheat genotypes used for heat tolerant analysis

Sl. No.	Genotype	Source	Salient features (Identified by Jaya, 2016)
1	HD3090	IARI, New Delhi	Check for irrigated late sown, higher leaf waxiness
2	HD2864	IARI, New Delhi	Timely sown, irrigated condition, low canopy temperature and high spikelets per spike
3	HI977	IARI RS, Indore	Check for irrigated late sown, high thousand grain weight under late sown.
4	DBW14	DWR, Karnal	Heat tolerant based on HSI
5	2 nd WYCYT41	CIMMYT, Mexico	Heat tolerant based on HTI
6	UASBW 10453	UAS, Dharwad	Heat tolerant based on HTI
7	HI8730	IARI RS, Indore	Irrigated, timely sown and more number of grains per spike
8	2 nd WYCYT10	CIMMYT, Mexico	High chlorophyll content
9	SATYN 9427	CIMMYT, Mexico	High chlorophyll content and more number of grains per spike
10	QCSN 35	CIMMYT, Mexico	Heat susceptible, high yielding under timely sown condition

Table 2: Details of markers associated with heat adaptive traits used in the present investigation

Sl. No.	Marker name	Chromo. No.	Sequence forward (5' to 3')	Sequence reverse (3' to 5')	Trait	References
1	<i>Xgwm210</i>	2A, 2B	TGCATCAAGAA TAGTGTGGAAG	TGAGAGGAAG GCTCACACCT	Flag leaf temperature depression	Mohamed <i>et al.</i> (2013)
2	<i>Xwmc9</i>	7A	AACTAGTCAAA TAGTCGTGTCCG	GTCAAGTCATCT GACTTAACCCG	Membrane stability and injury index	Roder <i>et al.</i> (1998)
3	<i>Xcfa2129</i>	1A	GTTGCACGAC CTACAAAGCA	ATCGCTCAC TCACTATCGGG	HSI kernel weight of main spike	Kumar <i>et al.</i> (2013)
4	<i>Xwmc661</i>	2B	CCACCATGGT GCTAATAGTGTC	AGCTCGTAACG TAATGCAACTG	Flag leaf temperature depression	Mohamed <i>et al.</i> (2013)
5	<i>Xwmc500</i>	1B	ATAGCATGTTG GAACAGAGCAC	CTTAGATGCAA CTCTATCCGGT	Flag leaf temperature depression	Mohamed <i>et al.</i> (2013)
6	<i>Xwmc361</i>	2B	AATGAAGATGC AAATCGACGGC	ATTCTCGCACT GAAAACAGGGG	Flag leaf temperature depression	Mohamed <i>et al.</i> (2013)
7	<i>Xbarc10</i>	2B, 4B, 5A, 7B	GCGTGCCACTGTA ACCTTTAGAAGA	GCGAGTTGGAATTA TTTGAATTAACAAG	Flag leaf temperature depression	Mohamed <i>et al.</i> (2013)
8	<i>xbarc49</i>	7A	GTCCACCAAAA TTAACAGCTCCTA	AGGCGCAGTGCT CGAAGAATATTAT	Thermal membrane depression	Talukder <i>et al.</i> (2014)
9	<i>Xgwm111.2</i>	2B	ACCTGATCAG ATCCCCATCG	TCTGTAGGCT CTCTCCGACTG	HSI kernel weight HSI kernel number	Kumar <i>et al.</i> (2013)
10	<i>Xgwm169</i>	6A	ACCACTGCAGA GAACACATACG	GTGCTCTGCT CTAAGTGTGGG	Flag leaf temperature depression	Mohamed <i>et al.</i> (2013)
11	<i>Xwmc407</i>	2A	CATATTTCCAA ATCCCCAACTC	GGTAATTCTAGG CTGAATATGCTC	Grain-filling duration	Kumar <i>et al.</i> (2013)
12	<i>Xbarc55</i>	1B	GCGGTCAACACA CTCCACTCCTCTCTC	CGCTGCTCCCAT TGCTCGCCGTTA	Chlorophyll content	Mohamed <i>et al.</i> (2013)
13	<i>Xbarc78</i>	4A	CTCCCCGGTCA AGTTTAATCTCT	GCGACATGGGAATT TCAGAAGTGCCTAA	Chlorophyll content	Mohamed <i>et al.</i> (2013)
14	<i>Xgwm276</i>	7B	ATTTCCTGA AGAAAAATATT	AATTTCACCTG CATACACAAG	Membrane stability	Roder <i>et al.</i> (1998)
15	<i>Xbarc32</i>	5B, 7B	GCGTGAATCCGGA AACCCTAATCTGTG	TGGAGAACTTCG CATTGTGTCATTA	HSI kernel weight of main spike	Barakat <i>et al.</i> (2011)
16	<i>Xgwm-132</i>	6B	TAC CAA ATCGA A ACA CAT CAG G	CAT ATC AAG GTC TCC TTC CCC	Grain filling rate	Barakat <i>et al.</i> (2011)
17	<i>Xbarc183</i>	2B	CCCGGGACCA CCAGTAAGT	GGATGGGGAATT GGAGATACAGAG	Flag leaf temperature depression	Mohamed <i>et al.</i> (2013)
18	<i>Xwmc603</i>	7A	ACAAACGGTGA CAATGCAAGGA	CGCTCTCTCG TAAGCCTCAAC	Membrane stability and injury index	Roder <i>et al.</i> (1998)
19	<i>Xwmc182</i>	7A	GTATCTCACGA GCATAACACAA	GAAAGTGTATG GATCATTAGGC	Membrane stability	Roder <i>et al.</i> (1998)
20	<i>xgwm291</i>	5A	AATGGTATCT ATTCCGACCCG	CATCCCTAG GCCACTCTGC	HSI kernel weight	Barakat <i>et al.</i> (2011)

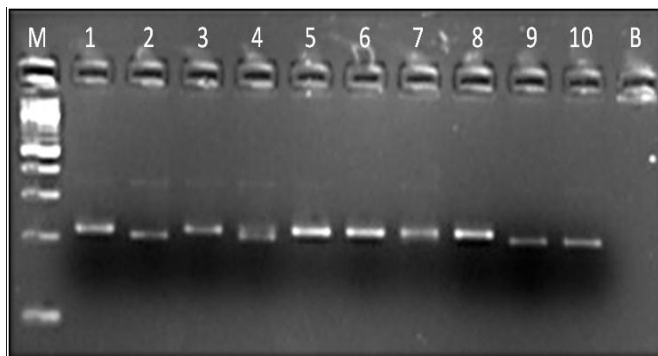


Fig. 1: Genotyping of ten bread wheat genotypes using *xbarc49* SSR marker

M= 100 bp ladder, 1: HD3090, 2: HD2864, 3: HI977, 4: DBW14, 5: 2nd WYCYT41, 6: UASBW10453, 7: HI8730, 8: 2nd WYCYT10, 9: SATYN9427 and 10: QCSN35

Results and Discussion

The current study aimed to assess wheat genotypes for drought tolerance through the evaluation of both agronomical traits and molecular markers. A diverse array of genotypes was analyzed, revealing promising candidates for breeding purposes.

The percentage reduction in yield under timely sown (control) and late sown (heat stress) conditions was calculated to assess trait sensitivity to heat stress (Table 3). Grain yield exhibited the highest sensitivity, with an 84.19% reduction due to heat stress. This reduction in yield was influenced by changes in several traits, including the number of grains per spike, number of tillers per meter, number of spikelets per spike, thousand-grain weight (g), and kernel weight per spike (g). Traits such as the number of tillers per meter, spikelets per spike, thousand-grain weight (g), and kernel weight per spike (g) were observed to be more sensitive to heat stress, while spike length (cm) and grains per spike showed less sensitivity.

Early maturity plays a crucial role in heat stress tolerance mechanisms. While initial crop growth remained unaffected, a shorter grain filling duration (GFD) resulted in accelerated crop maturity, leading to reduced seed set and thousand-grain weight, thereby significantly impacting grain yield Talukder *et al.* (2014). Grain filling duration exhibited considerable sensitivity to heat stress.

The results revealed that under the late sowing condition, grain yield exhibited high values of Phenotypic Coefficient of Variation (PCV) at 34.01% and Genotypic Coefficient of Variation (GCV) at 30.66% (Table 4). Similarly, under timely sown conditions, grain yield also displayed high PCV

(39.04%) and GCV (36.46%) values. These findings suggest that the variability observed in grain yield is less affected by environmental factors, indicating its suitability for early-generation selection. Similar observations of high GCV and PCV estimate for grain yield per plant was reported by Mohanty *et al.* (2016) and Elbashier *et al.* (2019).

Heritability is a useful quantitative parameter, which uses the role of heredity and environment in determining the expression of a trait (Allard 1960). Effective selection can be achieved only when additive effects are substantial and environmental effects are small. For grain yield, the magnitude of heritability was high (81.26 %) with high GAM (56.94 %) under late sown conditions (Li *et al.* 2019). Under timely sown conditions also heritability was high (87.21 %) coupled with high GAM (70.14 %) emphasizing that the additive genetic variation was the major component of genetic variation in the inheritance of these traits.

Expected genetic advance indicates the expected genetic progress for a particular trait under the selection cycle and measures the extent of its stability under selection pressure. Heritability estimates along with genetic advances are normally more helpful in predicting the gain under selection than heritability estimates alone in selecting the best individuals (Johnson *et al.*, 1955). High heritability along with high genetic advance was noticed for leaf waxiness, membrane stability index, days to 50 per cent flowering, grains per spike, thousand grains weight, kernel weight per spike and grain yield per plot under both environmental conditions (Table 4). These traits are the most important quantitative traits to be taken into consideration for effective selection in wheat. Similar results have been reported by Shankarrao *et al.* (2010), Majumder *et al.* (2008) and Yousafali *et al.* (2008).

Greenness is also an important physiological trait, which may be used to investigate and predict wheat yield potential under terminal heat stress. There was a high rate of variation among the tested genotypes. High heritability along with moderate genetic advance was noticed for NDVI at all three stages. In response to drought stress, the tolerant plant species can increase their chlorophyll contents, to avoid the possible negative effects resulting from the oxidative stress (Gill *et al.* 2017; Miransari and Smith 2019).

Stomatal conductance diminishes in high solar radiation and dry conditions, and a deficiency in soil moisture lowers the usual transpiration rate, which raises canopy temperature. (Rebetzke *et al.*, 2013).

Thus, canopy temperature can be used to study drought and heat tolerance in plants. The canopy temperature exhibited consistently low variability across critical growth stages, including booting, anthesis, and grain filling stages, in both timely and late sown conditions, indicating its sensitivity to heat stress. These findings, supported by moderate to high heritability, which underline the significant influence of the environment on this trait's expression. Singh *et al.* (2013) also reported similarly low variability for canopy temperature. However, heritability was notably high across stages, except during anthesis under late sowing conditions, consistent with findings by Akhilesh *et al.* (2014).

Using several tolerance indices for identifying tolerant genotypes provides valuable information and improves the precision in classifying the genotypes. Stress tolerance index in wheat was used in various research to select genotypes that could withstand high temperatures Aberkane *et al.* (2021) and Puri *et al.* (2015). Among the various heat indices available, heat susceptibility index (HSI) and heat tolerance index (HTI) were employed in determining the tolerance of the bread wheat genotypes as well as crosses based on their performance under the two different situations. Upon evaluating the heat susceptibility index (HSI), it was determined that certain genotypes, namely HD2864, DBW14, and HI8730, exhibit a tolerant response to heat stress, while SATYN9427 demonstrates a moderate level of tolerance. Conversely, genotypes such as HD3090, HI977, 2nd WYCYT41, UASBW10453, 2nd WYCYT10, and QCSN35 are classified as susceptible. However, when considering the heat tolerance index (HTI), only genotype 2nd WYCYT41 is categorized as tolerant, while the remaining genotypes are deemed susceptible. Similar studies have been reported by Thakur *et al.* (2019) and Bhardwaj *et al.* (2017).

Various studies have explored the application of SSR markers associated with heat stress in wheat (Rahimi *et al.*, 2019). Under late sown conditions, eight markers displayed significant associations with various traits (Table 7) Marker *Xbarc183*, previously linked with flag leaf temperature depression (Barakat

et al., 2011), exhibited associations with traits like days to 50% flowering, number of productive tillers per meter, grains per spike, and grain filling duration, with the highest contribution (R^2 value of 0.715) in the current study. Plant height was linked to markers *Xbarc49* and *Xbarc55*, with *Xbarc49* associated with osmotic potential (Ilyas *et al.*, 2020) and *Xbarc55* linked to chlorophyll content (Mohamed *et al.*, 2013).

Two critical yield attributes, spikelets per spike and grains per spike, under late sown conditions were associated with marker *Xwmc9*, while Roder *et al.* (1998) reported its association with membrane stability index. Marker *Xwmc661*, previously associated with flag leaf temperature depression (Mohamed *et al.*, 2013), was linked to grain-filling duration and membrane stability at grain grain-filling stage in this study.

Canopy temperature, a crucial indicator of heat stress sensitivity, CT-I and CT-III displayed associations with markers *Xwmc407*, which also showed association with NDVI at the booting stage. On the other hand, *Xwmc407* has shown association with grain filling duration (Kumar *et al.*, 2016) and for super oxide dismutase (Ilyas *et al.*, 2020). Marker *Xwmc603*, previously associated with membrane stability and injury index (Roder *et al.*, 1998), was found to be linked with leaf waxiness under heat stress conditions in this study.

Under timely sown conditions, five markers (*Xbarc183*, *Xwmc661*, *Xbarc49*, *Xgwm276*, and *Xwmc9*) demonstrated associations with various morpho-physiological traits (Table 6). T-tests were conducted based on allelic differences obtained from markers associated with heat-adaptive traits compared to the mean performances of genotypes. Eight markers, previously associated with 12 heat-adaptive traits in Single Marker Analysis (SMA) under late-sown conditions, were considered for the T-test. Among the 12 agronomic and physiological traits, significant differences between genotypes were observed for eight traits in terms of their allelic differences (Table 8).

Table 3: Per cent reduction for traits between the timely sown and late sown conditions of experiment

Sl No.	Characters	TS	LS	Per cent reduction
1	DFP	56.26	55.93	0.59
2	DM	98.96	93.30	6.07*
3	PH	75.23	70.80	6.26
4	TM	99.76	90.60	10.11*
5	SL	9.71	9.30	4.41
6	SPS	19.16	16.70	14.73*
7	GPS	49.00	46.33	5.76

8	TGW	35.17	30.68	14.63*
9	GY	34.02	18.47	84.19*
10	KWPS	1.56	1.44	8.33
11	GFD	32.33	26.70	21.09*
12	SPAD I	50.30	48.50	3.71
13	SPAD II	52.51	49.81	5.42*
14	SPAD III	43.76	38.76	12.90*
15	CT I	29.34	29.50	-0.54
16	CT II	28.92	31.54	-8.31*
17	CT III	31.02	32.07	-3.27
18	NDVI I	0.63	0.54	16.67*
19	NDVI II	0.58	0.50	16.00*
20	NDVI III	0.49	0.40	22.50*
21	LW	6.14	6.44	-4.66
22	MSI-I	52.22	41.61	25.50*
23	MSI-II	61.70	52.88	16.68*

*Significance at the 5% probability **. Significant at the 1% probability

Table 4: Genetic variability parameters for morpho-physiological characters in parents grown under timely and late sown condition.

Sl. No.	Characters	Mean		Range		GCV (%)		PCV (%)		H ² (%) BS		GAM (%)	
		TS	LS	TS	LS	TS	LS	TS	LS	TS	LS	TS	LS
1	SPAD-I	50.30	48.50	47.60-54.60	43.80-53.40	4.76	5.31	5.33	6.93	79.80	58.70	8.77	8.37
2	SPAD-II	52.51	49.81	49.03-56.13	46.00-54.13	3.43	4.94	4.79	7.15	51.40	47.70	5.08	7.03
3	SPAD-III	43.76	38.76	37.73-46.73	36.06-42.33	5.80	4.38	6.51	6.64	79.40	43.50	10.66	5.94
4	Canopy temperature –I	29.34	29.50	26.63-32.33	27.33-31.20	5.57	4.66	6.63	5.21	70.80	79.90	9.68	8.58
5	Canopy temperature –II	28.92	31.54	26.63-33.23	28.60-33.56	7.86	4.09	9.09	5.45	74.70	56.40	13.99	6.33
6	Canopy temperature –III	31.02	32.07	28.20-33.56	29.83-33.70	5.87	3.96	6.65	4.57	78.10	75.00	10.69	7.07
7	NDVI-I	0.62	0.54	0.56-0.68	0.43-0.64	6.37	10.05	8.10	11.24	61.90	79.90	10.32	18.51
8	NDVI-II	0.58	0.50	0.49-0.64	0.45-0.56	7.56	7.89	8.20	9.35	85.00	71.10	14.37	13.70
9	NDVI-III	0.49	0.40	0.45-0.54	0.37-0.45	6.90	6.13	8.61	7.97	64.00	59.00	11.40	9.70
10	Leaf waxiness	6.14	6.44	4.50-7.40	4.20-7.80	17.34	20.23	17.47	20.34	98.60	99.00	35.48	41.48
11	Membrane stability index- I	52.22	41.61	43.57-61.05	36.66-52.24	9.13	10.54	9.47	10.89	93.00	93.70	18.15	21.02
12	Membrane stability index- II	61.70	52.88	47.77-73.19	44.92-60.38	10.74	9.68	10.95	9.97	96.20	94.20	21.71	19.35
13	Grain filling duration	32.33	26.70	27.00-37.00	21.66-33.33	9.89	14.38	10.57	14.53	87.60	98.00	19.07	29.32
14	Days to 50 per cent flowering	56.26	55.93	48.66-61.66	48.33-61.66	10.21	10.33	10.47	10.52	95.10	96.30	20.52	20.89
15	Days to maturity	98.96	93.30	91.33-103.66	85.00-98.00	4.12	4.53	4.21	4.64	95.60	95.10	8.30	9.10
16	Pant height(cm)	75.23	70.80	67.00-85.00	63.66-79.33	7.41	6.22	7.75	7.05	91.40	77.70	14.60	11.30
17	Tillers per meter	99.76	90.60	90.33-107.66	76.00-104.66	6.38	9.24	6.90	10.53	85.40	77.00	12.15	16.71
18	Spike length (cm)	9.71	9.30	8.33-11.50	8.00-10.50	10.16	7.96	12.42	10.19	66.90	61.10	17.11	12.83
19	Spikelets per spike	19.16	16.70	16.00-21.66	14.00-19.00	9.77	7.43	10.76	9.75	82.50	58.00	18.29	11.66
20	Grains per spike	49.00	46.33	35.00-60.33	33.33-58.33	18.00	17.70	19.46	18.49	85.50	91.60	34.28	34.89
21	Thousand grain weight (g)	35.17	30.68	28.45-44.50	26.43-36.53	11.74	10.10	13.08	11.27	80.60	80.30	21.71	18.65
22	Kernel weight per spike (g)	1.56	1.44	1.05-2.08	0.95-1.90	23.79	24.32	25.77	25.69	85.00	89.00	45.23	47.42
23	Grain yield (q / ha)	34.02	18.47	19.09-60.91	10.08-26.83	36.46	30.66	39.04	34.01	87.21	81.26	70.14	56.94

TS= Timely sown, LS= Late sown

Table 5: Heat stress indices of parents for yield (q hac⁻¹) under study

Sl. No.	Parents	Ys	Yp	HSI	HTI
1	HD3090	22.19	36.30	0.85	0.70
2	HD2864	26.83	33.02	0.41	0.77
3	HI977	11.93	30.63	1.34	0.32
4	DBW14	18.77	19.09	0.04	0.31
5	2nd WYCYT41	26.64	60.91	1.23	1.40
6	UASBW 10453	17.26	47.97	1.40	0.72
7	HI8730	18.03	22.04	0.40	0.34
8	2nd WYCYT10	12.14	38.04	1.49	0.40
9	SATYN 9427	20.82	28.28	0.58	0.51
10	QCSN 35	10.08	23.93	1.27	0.21

HSI-Heat susceptibility index, HTI-Heat tolerance index, YS-Yield under stress and YP-Yield under non-stress.

Table 6: Marker Trait association detected in bread wheat genotypes under timely sown condition through single marker analysis

Sl. No.	Marker	Traits(TS)	Chromosome No.	F(1,N-2)	P (Value)	R ²
1	Xbarc183	DFF	2B	5.46	0.047*	0.405
		GFD		7.05	0.029*	0.468
		CT-II		17.48	0.003**	0.686
2	Xbarc49	PH	7A	8.56	0.019*	0.517
3	Xwm661	TM	2B	8.7	0.018*	0.521
		TGW		7.68	0.024*	0.490
		SPAD-II		5.91	0.041*	0.425
		CT-II		10.63	0.011*	0.571
4	Xgwm276	SL	7B	20.99	0.001**	0.724
		SPAD-III		6.97	0.029*	0.465
5	Xwmc9	GPS	7A	7.86	0.023*	0.495
		KWPS		7.26	0.027*	0.475

*Significance at the 5% probability

**Significant at the 1% probability

Table 7: Marker Trait association detected in bread wheat genotypes under late sown condition through single marker analysis and T-test

Sl. No.	Marker	Traits(TS)	Chromosome No.	F(1,N-2)	P (Value)	R ² (%)	T-test* (<0.05= Alpha value)
1	Xbarc183	DFF	2B	5.40	0.048*	0.403	0.06
		TM		9.08	0.016*	0.531	0.0002*
		GPS		5.32	0.049*	0.399	0.0004*
		GFD		20.12	0.002**	0.715	0.003*
2	Xbarc49	PH	7A	8.09	0.021*	0.502	0.021*
3	Xbarc55	PH	1B	7.67	0.024*	0.489	0.003*
4	Xwmc9	SPS	7A	5.96	0.041*	0.427	0.006*
		GPS		6.57	0.033*	0.450	0.0004*
5	Xwm661	GFD	2B	11.17	0.011*	0.582	0.003*
		MSI-II		14.06	0.005**	0.637	0.003*
6	Xgwm169	SPAD-III	6A	5.93	0.041*	0.425	0.0007*
7	Xwmc407	CT-I	2A	6.25	0.036*	0.438	1.54
		CT-III		5.66	0.044*	0.414	1.38
		NDVI-I		5.53	0.046*	0.408	0.013*
8	Xwmc603	LW	7A	6.19	0.037*	0.436	0.001*

*Significance at the 5% probability **. Significant at the 1% probability

Table 8: Details of genotypes carrying different markers associated with different heat adaptive traits

Sl. No.	Genotype	Markers	Heat adaptive traits (w.r.t. markers)
1	HD3090	<i>xbarc183</i> , <i>xgwm169</i> and <i>xwmc603</i>	SPS, GPS, SPAD-III and LW
2	HD2864	<i>xbarc183</i>	TM, GPS and GFD
3	HI977	<i>xbarc183</i> and <i>xwmc9</i>	TM, GPS, SPS and MSI-II
4	DBW14	<i>xwm661</i> , <i>xgwm169</i> and <i>xwmc407</i>	TM, MSI-II, SPAD-III and NDVI- I
5	2 nd WYCYT41	<i>xwmc407</i> and <i>xgwm169</i>	SPAD-III, NDVI- I and MSI-II
6	UASBW 10453	<i>xwm661</i> and <i>xwmc603</i>	MSI-II, LW
7	HI8730	<i>xbarc183</i> , <i>xwmc603</i> and <i>xwmc407</i>	TM, GFD, LW and NDVI- I
8	2 nd WYCYT10	<i>xwm661</i> and <i>xwmc603</i>	MSI-II and LW
9	SATYN 9427	<i>xbarc183</i> , and <i>xwmc603</i>	TM, GFD and LW
10	QCSN 35	<i>xwm661</i>	MSI-II

Conclusion

From the present study, it is evident that the genotypes studied can provide a good source of material for a further breeding program in the selection of genotypes for heat tolerance. Consequently,

information on the genetic parameters such as genetic and phenotypic coefficient of variation, heritability and genetic advance can help the breeder to evolve suitable cultivars within a short time. The knowledge of the heritability of traits helps decide the selection

procedure to be followed to improve the trait in a particular situation. SSR markers found to be associated with heat adaptive traits have utility beyond merely categorizing wheat genotypes; they can also aid in the pyramiding of heat adaptive traits in a single genetic background. By crossing genetically distant genotypes, more diverse populations can be created, offering opportunities to enhance genetic variation across different chromosomal locations and traits, as suggested by Abbasov (2018). Moreover, our findings demonstrated that the morphological and molecular analysis could be useful for describing wheat genetic variation of heat tolerance.

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