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RESEARCH ARTICLE

Validation of SSR markers linked to heat tolerance in bread wheat genotypes

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Abstract

Parental selection and breeding rely on the existence of genetic diversity and relationships present in germplasms. The objective of this study was to assess the molecular divergence for terminal heat stress tolerance in twenty wheat genotypes, utilizing 25 SSR markers associated with heat tolerance, which are linked to the trait of interest across various chromosomes. For molecular diversity assessment, fourteen polymorphic SSR markers, out of the twenty-five, amplified a total of 33 alleles, which were distributed across nine chromosomes. The number of alleles per locus ranged from 2 to 3, with an average of 2.36 alleles per locus. Polymorphism information content (PIC), resolving power (Rp), effective multiplex ratio (EMR) and marker index (MI) values were high for markers *viz.*, Xwmc603, Xwmc161 and Xgwm577, indicating that they had the most discriminatory power. Based on Jaccard's similarity coefficient, the genetic similarity among the 20 wheat genotypes ranged from 0.36 to 0.88. The dendrogram generated from UPGMA cluster analysis revealed two primary clusters: cluster I, which comprised three genotypes and cluster II, which included seventeen genotypes. Genotypes GS/2019-20/6046, HPYT-2019-20/416 and GS/2019-20/5042 fall in cluster I and the remaining seventeen genotypes are included in cluster II, with similarity coefficient values ranging from 0.70 to 0.79 and 0.36 to 0.88, respectively. The current study demonstrated that there is enough variation present among the genotypes at the molecular level and the findings would help to make use of diverse genotypes as parents in future hybridizing programs to improve terminal heat tolerance.

Keywords: dendrogram; genetic diversity; terminal heat tolerance; UPGMA

Introduction

Wheat (*Triticum aestivum* L. em Thell) is one of the oldest domesticated crops and the world's second most significant cereal after rice in terms of cultivation and consumption. In contrast, global cereal production, ranks second after maize, with an estimated 793.24 million metric tons harvested in the 2024/2025 period (1). It is extensively cultivated in India across a diversified agro-climatic condition, spanning from the northern to southern highlands and from Gujarat to Assam. It is well-suited to cooler environmental conditions, which is why it is generally cultivated during the cooler winter months in subtropical and tropical regions. Temperature is a key natural factor that influences the rate of crop development through various physiological mechanisms. Heat stress is not limited to specific regions; instead, rising temperatures harm worldwide wheat output (2). Wheat crops are highly vulnerable to heat stress, especially during the grain filling phase.

Late sowing is frequent in rice-wheat growing zones across India. Even a 1°C rise in mean temperature can reduce grain production, potentially leads to significant losses, with wheat yields in India projected to decline by up to 50% if exposed to 32-38°C during the critical grain formation stage, based on an estimated 3-4% loss per 1°C increase above 15-20°C (3-5). Heat stress adversely affects wheat development by impairing enzyme activation, chlorophyll biosynthesis and photosynthesis, leading to disruptions in heading, flowering, grain filling and aging (6). Despite its importance, the genetic basis of terminal heat tolerance in wheat remains little understood (7). Consequently, identifying genetically diverse genotypes with the ability to withstand high temperatures during the grain filling stage, preventing yield reduction. It is crucial for breeding programs to develop wheat varieties that can withstand terminal heat stress.

Genetic diversity is crucial for the success of any crop breeding initiative. The existing heterogeneity among

germplasm can be assessed using a variety of morphological and genetic markers. Molecular genome analysis at the DNA level offers significant advantages, as DNA sequences remain consistent across all living cells of a plant, regardless of its physiological or developmental state. Classical breeding methods can be time-consuming when evaluating quantitative traits, underscoring the need for molecular markers to enhance research efforts. PCR-dependent co-dominant marker-based profiling technologies have become essential tools in genetic diversity analysis due to their high levels of polymorphism and ease of analysis. Scientists can employ these technologies to evaluate the extent of genetic diversity, characterize genetic resources and identify markers linked to tolerance. These practical approaches are helpful for developing improved cultivars that are tolerant to abiotic stresses and for effectively conserving germplasm in stress-prone areas. Integrating molecular markers into breeding programs expedites the selection process, allowing breeders to more efficiently create stress-tolerant cultivars.

Simple sequence repeats (SSRs), commonly known as microsatellites, are popular molecular markers for assessing genetic diversity in populations because of their locus specificity, co-dominance and multi-allelic nature, excellent reproducibility, as well as significant polymorphism. Additionally, they are easily assayed and are broadly distributed throughout the genome, with potential for automated analysis (8-10). All of these characteristics make it an ideal marker for genetic characterization in wheat (11, 12). Therefore, the current study aimed to assess molecular divergence between genotypes for heat tolerance using SSR and functional markers specific to different chromosomes.

Materials and Methods

Experimental plant material

Initially, 48 diverse genotypes were obtained from the pre-breeding program of the Wheat Research Station, Navsari Agricultural University, Bardoli, Gujarat, India, focused on heat tolerance. From this, 20 genotypes (Table 1) under heat stress were selected for subsequent breeding trials. The selection of these 20 genotypes was based on morphological, physiological and quality traits assessments related to high-temperature stress tolerance conducted during field trials at two different sowing times: optimum and late sowing. Crops planted on 29th November, 2021, were considered optimum sowing, while those sown on 6th January, 2022, were classified as late sowing.

Genotyping with SSR markers

DNA isolation

The seeds of 20 genotypes were grown in 2L capacity polyethylene pots under a glasshouse at N. M. College of Agriculture, Navsari Agricultural University, Navsari, Gujarat, India. After five weeks of planting, young and fresh leaves were picked and DNA was isolated using the CTAB (Cetyl Trimethyl Ammonium Bromide) extraction method (13) with some modifications. This procedure was performed at the Molecular Breeding Laboratory of the Department of Genetics and Plant Breeding, N. M. College of Agriculture, NAU, Navsari.

Determination of DNA concentration and purity

The purity of extracted DNA was assessed by placing 1.5 µl of DNA into the Nanodrops spectrophotometer (Thermo, USA). The DNA concentration and absorbance were measured at 260 and 280 nm. To assess DNA quality, the A260/A280 ratio was calculated and concentration was measured in ng/µl using absorbance readings. Pure DNA has an A260/A280 ratio between 1.8 and 2.0. The DNA purity and concentration of different wheat genotypes are presented in Table 1.

Primers used in this study

Twenty-five SSR markers (Wheat Microsatellite Consortium (WMC), X Göttingen Wheat Microsatellite (Xgwm), Conserved Fragment Derivatives (cfd) and X Beltsville Agricultural Research Center (Xbarc) series) were chosen based on their highly polymorphic and allele-specific characteristics associated with heat tolerance traits, as previously described by various researchers. All of these primers were custom-manufactured by G-Biosciences in the United States. Table 2 shows the chromosome locations, base sequences of forward and reverse primers

Table 1. List of experimental genotypes with purity and concentration of DNA

Sr. No.	Genotype	Absorbance ratio A260/A280	Concentration (ng.µl^{-1})
1	GS/2019-20/6046	1.90	2075.7
2	GS-2018-19/1007	1.57	2328.2
3	SAWYT-2018-19/309	1.95	1284.2
4	HPYT-2019-20/416	1.89	2216.2
5	GS/2019-20/5042	1.86	2003.2
6	HTWYT/2019-20/30	1.84	1420.2
7	EHT-2018-19/443	1.78	1939.8
8	HTWYT/2019-20/17	1.97	1528.1
9	GW 499	1.86	1939.6
10	GS/2019-20/7004	1.89	1721.8
11	EHT-2018-19/406	1.84	2469.3
12	GS/2018-19/6027	1.86	1571.6
13	GW 173	1.82	1389.3
14	HTWYT/2019-20/39	1.80	1721.1
15	HI 1628	1.88	2015.9
16	EHT-2018-19/403	1.90	2039.9
17	GS/2018-19/4049	1.98	1509.4
18	GS/2019-20/4003	2.01	1753.4
19	GS/2019-20/3060	1.92	3096.1
20	HTWYT/2018-19/36	2.04	1172.9

and their annealing temperatures. These markers were utilized to evaluate the genetic diversity of the 20 genotypes.

Amplification of SSR Primers

The PCR reaction volume for SSR primers was set to 10 µl. Amplification was conducted using a thermal cycler (BIO-RAD, USA), with the cycle settings outlined in Table 3. A 100-bp ladder was used to assess the size of the PCR products. Gel electrophoresis of amplified products was performed on a 3% agarose gel dyed with Green Stain (0.5 µg/mL) at 90 V for 2 hr and 30 min. The gel was visualized using the Gel Doc™ XR Imaging system under UV light.

Analysis of SSR marker data:

Data were noted in a binary format, indicating the absence (0) or presence (1) of bands across the amplified profile generated by a primer (14). The binary data were utilized to quantify genetic similarity using Jaccard's coefficient (15) and an Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrogram was created to evaluate the genetic link between genotypes. All statistical analyses were carried out using NTSYSpc computer genotypes, including cluster analysis utilizing the SHAN module of NTSYSpc version 2.0. The polymorphism information content (PIC) value was determined in agreement with (16).

$$PIC_{(Codominant\ marker)} = 1 - \sum P_i^2 \qquad \text{Eqn. 1}$$

where P = Frequency of present allele

PIC value ranged from 0 to 1 for codominant markers.

Each primer's resolving power (Rp) was estimated using the formula

$$R_p = \sum Ib \qquad \text{Eqn. 2}$$

where Ib (band informativeness) equals .

$$1-[2x\ (0.5 - p)]$$

Where p represents the proportion of different wheat genotypes that possess that band (17).

The efficiency of the marker ratio was calculated by multiplying the total number of polymorphic loci for each primer by the proportion of polymorphic loci relative to the total number of loci, using the formula

$$EMR = np\ X \left(\frac{np}{n}\right) \qquad \text{Eqn. 3}$$

where "np" represents the number of polymorphic loci and "n" indicates the total number of loci. DNA markers are more effective when the EMR (Effective multiplex ratio) value is more significant (18). Marker index (MI) values are derived using the equation (19)

$$Mi = PIC\ X\ EMR \qquad \text{Eqn. 4}$$

Results and Discussion

Simple sequence repeat marker Informative

A total of 25 SSR markers were utilized to screen the 20 wheat genotypes for high temperature tolerance. Out of the 25 SSR markers used, 23 showed obvious amplification patterns. Among amplified primers, 14 were found to be polymorphic and amplified a total of 33 alleles with a high polymorphism (~100 percent) distributed among nine chromosomes. Amplification pattern of 20 wheat genotypes obtained by SSR markers (Xgwm 261 and Xgwm 95) presented in Fig. 1 and Fig. 2. Several criteria were employed to evaluate the informativeness of SSR markers, including the number of alleles, polymorphic information content (PIC), resolving power (Rp), effective multiplex ratio (EMR) and marker index (MI) (Table 4).

The number of alleles per locus varied between two and three (Table 4). The limited number of alleles revealed a narrow genetic base in the three wheat cultivars (20). The variation in the number of alleles per locus may be attributed to differing locus-specific mutation rates and reflects significant differences in allelic diversity among SSR loci (21). The average number of alleles per locus (2.36) for the 20 genotypes is comparable to a previous study that reported an average of 2.585 alleles per locus in 44 wheat genotypes using 100 SSR markers (22). Furthermore, higher mean allele counts of 13 and 5.9 were observed in 40 and 10 wheat genotypes, respectively (23, 24). It is essential to approach comparisons of allelic diversity reported in other studies with judiciousness, considering the differences in sample sizes used.

The PIC value, ranging from 0.32 (Xgwm 148) to 0.80 (Xwmc 658), with a mean of 0.54 as shown in Table 4, serves as a valuable metric for assessing the informativeness of molecular markers and their ability to detect genetic diversity. Because the PIC value exceeds 0.5, the marker is highly informative and effective in detecting genetic

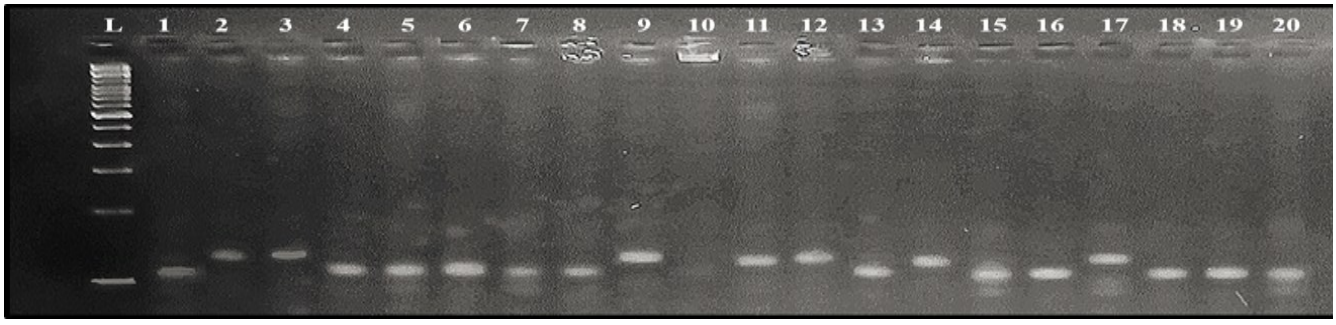

Fig. 1. SSR profile of 20 wheat genotypes generated by using primer Xgwm 261

Table 2. List of primers used to study heat tolerance in wheat genotypes

Sr. No	Maker	Position	Forward Primer	Reverse Primer	Ta (°C)	References
1	Xgwm448	2A	AAACCATATTGGGAGGAAAGG	CACATGGCATCACATTTGTG	53.5	(25)
2	WMC24	1A	GTGAGCAATTTTGATTATACTG	TACCCTGATGCTGTAATATGTG	51.0	(33)
3	Xwmc48	4B	GAGGGTTCTGAAATGTTTTGCC	ACGTGCTAGGGAGGTATCTTGC	55.0	(34)
4	Xgwm186	5A	GCAGAGCCTGGTTCAAAAAG	CGCCTCTAGCGAGAGCTATG	55.0	(34)
5	Xwmc364	7B	ATCACAATGCTGGCCCTAAAAC	CAGTGCCAAAATGTCGAAAGTC	55.5	(35)
6	Xgwm95	2A	GATCAAACACACACCCCTCC	AATGCAAAGTGAAAAACCCG	58.7	(35)
7	Xwmc273	7A	AGTTATGTATTCTCTCGAGCCTG	GGTAACCACTAGAGTATGTCCTT	55.6	(35)
8	Xgwm148	2B	GTGAGGCAGCAAGAGAGAAA	CAAAGCTTGACTCAGACCAAA	59.1	(35)
9	Xgwm11	7B	GGATAGTCAGACAATTCTTGTG	GTGAATTGTGTCTTGTATGCTTCC	60.0	(36)
10	Xgwm293	5A	TACTGGTTCACATTGGTGCG	TCGCCATCACTCGTTCAAG	55.2	(36)
11	Xwmc658	2A	CTCATCGTCCTCCTCCACTTTG	GCCATCCGTTGACTTGAGGTTA	59.1	(37)
12	Xgwm 05	3A	GCCAGCTACCTCGATACAACTC	AGAAAGGGCCAGGCTAGTAGT	57.5	(37)
13	Xbarc121	7A	ACTGATCAGCAATGTCAACTGAA	CCGGTGTCTTTCCTAACGCTATG	61.0	(38)
14	Xwmc161	4A	ACCTTCTTTGGGATGGAAGTAA	GTACTGAACCACTTGTAACGCA	56.0	(38)
15	Xgwm577	7B	ATGGCATAATTTGGTGAAATTG	TGTTTCAAGCCCAACTTCTATT	59.5	(38)
16	Xgwm335	5B	CGTACTCCACTCCACACGG	CGGTCCAAGTGCTACCTTTC	59.7	(39)
17	cfd43	2D	AACAAAAGTCGGTGCAGTCC	CCAAAAACATGGTTAAAGGGG	54.0	(39)
18	Xgwm261	2D	CTCCCTGTACGCCTAAGGC	CTCGCGCTACTAGCCATTG	57.0	(39)
19	Xwmc527	3B	ACCCAAGATTGGTTGCAGAA	GCTACAGAAAACCGGAGCCTAT	55.8	(39)
20	Xwmc603	7A	ACAAACGGTGACAATGCAAGGA	CGCCTCTCTCGTAAGCCTCAAC	57.0	(40)
21	Xbarc113	6A	GCGCACAACAACGGACACTTAACAATT	GGGACTCATTTAGCTTCTACTCGCCATTA	56.0	(41)
22	Xgwm337	1B	CCTCTTCCTCCCTCACTTAGC	TGCTAACTGGCCTTTGCC	56.0	(42)
23	Xgwm111	7D	TCTGTAGGCTCTCTCCGACTG	ACCTGATCAGATCCCACTCG	50.9	(42)
24	Xwmc479	7A	GACCTAAGCCCAGTGTCATCAG	AGACTCTTGGCTTTGGATACGG	55.8	(43)
25	Xgwm190	1B	GTGCTTGCTGAGCTATGAGTC	GTGCCACGTGGTACCTTTG	55.6	(44)

Table 3. PCR reaction conditions for SSR primers

Sr. No.	Step	Temperature (ºC)	Duration (Minutes)	No. of cycle
1	Initial Denaturation	94.0	5.00	1
2	Denaturation	94.0	0.30	
3	Annealing	50.9 -61.0	0.30	30
4	Extension	72.0	0.45	
5	Final extension	72.0	10:00	1
6	Halt	4.0	∞	-

Table 4. Summary of SSR amplified products

Primer	Position	Alleles (no.)	Band size	Rp	EMR	MI	PIC	QTL/ Trait	QTL/Trait Reported by
WMC 24	1A	3	145-170	2.20	3	1.10	0.37	GFR	(33)
Xgwm 95	2A	2	100-123	1.90	2	1.04	0.52	-	-
Xwmc 658	2A	2	138-205	1.20	2	1.60	0.80	-	-
Xbarc 121	7A	2	115-135	1.90	2	1.08	0.54	Thylakoid membrane damage	(38)
Xwmc 161	4A	3	150-180	2.00	3	1.94	0.65	-	-
Xgwm 448	2A	2	230-250	1.80	2	1.03	0.52	-	-
Xgwm 261	2D	2	170-201	1.80	2	1.15	0.58	HSI of the Single kernel weight of main spike	(39)
Xwmc 603	7A	3	80-110	1.90	3	2.06	0.69	-	-
Xgwm 577	7B	3	120-170	1.50	3	1.88	0.63	GFR	(38)
Xgwm 111	7D	2	130-140	1.90	2	1.04	0.52	LAUD	(42)
Xgwm 190	1B	2	210-230	1.90	2	0.98	0.49	Grain yield	(44)
Xgwm 148	2B	3	121-150	2.10	3	0.95	0.32	-	-
Xgwm 337	1B	2	190-203	2.20	2	0.78	0.39	LT	(42)
cfd 43	2D	2	200-245	1.50	2	1.14	0.57	GFD	(39)
Range	-	2 - 3	80 - 250	1.20 - 2.20	2 - 3	0.78 -2.06	0.32-0.80	-	-
Total	-	33	-	-	-	-	7.59	-	-
Average	-	2.36	-	1.84	2.36	1.27	0.54	-	-

Rp - Resolving power; EMR - Effective multiplex ratio; MI - Marker index; PIC - Polymorphism information content; QTL - Quantitative trait loci; GFR – Grain filling rate; HSI – Heat susceptible index; LAUD - Leaf area under development; LT – Leaf temperature; GFD – Grain filling duration

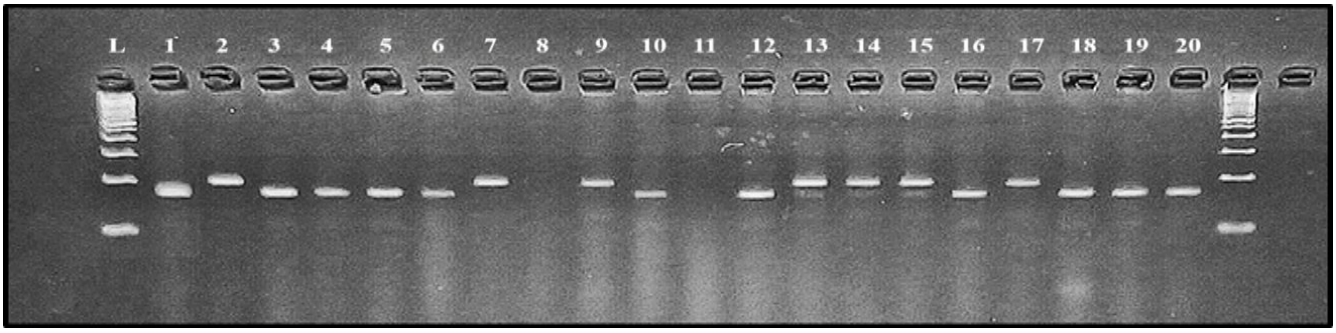

Fig. 2. SSR profile of 20 wheat genotypes generated by using primer Xgwm 95

1) GS/2019-20/6046	8) HTWYT/2019-20/17	15) HI 1628
2) GS-2018-19/1007	9) GW 499	16) EHT-2018-19/403
3) SAWYT-2018-19/309	10) GS/2019-20/7004	17) GS/2018-19/4049
4) HPYT-2019-20/416	11) EHT-2018-19/406	18) GS/2019-20/4003
5) GS/2019-20/5042	12) GS/2018-19/6027	19) GS/2019-20/3060
6) HTWYT/2019-20/30	13) GW 173	20) HTWYT/2018-19/36
7) EHT-2018-19/443	14) HTWYT/2019-20/39	**Ladder size** - 100 bp

diversity (25). In this study, about 71% of the SSR markers permeate distinct chromosomes of the A, B and D genomes by exhibiting a PIC value >0.50, indicating that the majority of markers facilitated a extreme level of polymorphism, with the exceptions for markers Xgwm 148, WMC 24, Xgwm 337 and Xgwm 190. The findings using SSRs suggest that they could be employed as a marker to aid in selection for heat stress tolerance through molecular plant breeding. The PIC values have been extensively used in previous genetic diversity assessments, with a study on 30 wheat genotypes reporting PIC values ranging from 0.21 to 0.23 using 10 markers (26). In comparison, another study with 16 genotypes and 60 markers reported a PIC value of 0.34 to 0.59 (27).

The size of the amplicons becomes crucial for identifying which fragments correspond to specific loci, especially when many of the microsatellite markers amplify complex, multilocus profiles (28). The size of the generated PCR products varied from 80 to 250 bp (Table 4). Fifteen microsatellite markers generated products from 77 to 266 bp in size in various wheat genotypes (29). Using 20 SSR markers on five Egyptian bread wheat cultivars and one wild wheat (30), an allelic size range from 59 to 635 bp was identified. However, polymerase slippage during repeat amplification is believed to contribute to the formation of fragments that are shortened by multiples of the repeat units (31).

The resolving power ranged between 1.20 (Xwmc 658) and 2.20 (WMC 24 and Xgwm 337), with a mean of 1.84. The RP index is used to determine the markers that recognize genotypes most efficiently and to offer a more precise estimate regarding the number of genotypes detected by a primer (17). Two SSR primers, Xgwm 337 and WMC24, obtained high RP values (2.20) and hence appear to be more informative primers for genotype differentiation. RP values varied from 7.2 (UBC-815) to 16.5 (UBC845), with a mean of 12 per primer (27). Marker index ranged from 0.78 (Xgwm 337) to 2.06 (Xwmc 603), with a mean of 1.27. A higher marker index indicates that the marker is more efficient in simultaneously analysing a larger number of bands (32). The Marker Index (MI) ranged from 0.41 to 3.36, with a mean of 1.34, based on 10 ISSR primers applied to 36 wheat accessions (27). The primers' effective multiplex ratio (EMR) ranged from 2.00 to 3.00, with a mean value of 2.36. Specifically, primers WMC 24, Xwmc 161, Xwmc 603, Xgwm 577 and Xgwm 148 recorded the highest EMR of 3, while the remaining primers exhibited an EMR of 2. These parameters are critical for evaluating the discriminatory capacity of molecular marker systems in various plant species. For instance, in wheat, ISSR markers have been reported to achieve an EMR of 5.69 (27).

Therefore, PIC, MI, EMR and Rp information could be used to evaluate the informativeness of the markers and their usefulness in germplasm characterization. High PIC, EMR, Rp and MI values *viz.*, Xwmc603, Xwmc161 and Xgwm577 indicated that, it had the most discriminatory power among the 25 tested primer The markers WMC 24 and Xgwm 577 (grain filling rate), Xbarc 121 (Damage of thylakoid membrane), Xgwm 261 (heat susceptible index (HSI) of single kernel weight of main-spike), Xgwm 111 (leaf area under development (LAUD)), Xgwm 190 (Grain yield), Xgwm 337 (leaf temperature (LT)) and cfd 43 (grain filling duration) were specific for heat tolerance because these markers already identified as gene specific and are linked with a different QTL those responsible for heat tolerance traits (Table 4).

Cluster analysis based on Jaccard's similarity coefficient

Using the similarity matrix, a dendrogram was constructed using NTYSISpc version 2.02's UPGMA cluster analysis approach, which was based on Jaccard's coefficient. Jaccard's similarity coefficients (Table 5) based on SSR markers were worked out and they range from 0.36 to 0.88 with an average of 0.67. The UPGMA dendrogram classified all wheat genotypes into two main distinct clusters *viz.*, cluster I and cluster II, in which maximum number of genotypes (17) fell into cluster II which were also genetically diverse amongst themselves (Fig. 3). Based on ISSR binary data, 30 wheat genotypes were categorized into five groups using the Jaccard similarity matrix's complete linkage approach (27).

Cluster-I comprised of three genotypes showing Jaccard similarity coefficient varied from 0.613 to 0.708 and was further separated into two sub-clusters A and B. Sub cluster-A composed of GS/2019-20/6046 and HPYT-2019-20/416 which showed (0.79) genetic similarity while, sub cluster-B consisted of GS/2019-20/5042 exhibited genetic similarity with GS/2019-20/6046 (0.73) and HPYT-2019-20/416 (0.70). Cluster-II was the largest and contained 17 genotypes, showing Jaccard's similarity coefficient value ranged from 0.36 to 0.88. It is interesting to note that cluster II was further separated into three subclusters, C, D and E, to simplify their comparative study. Seven genotypes, *viz.*, GS-2018-19/1007, GW 499, EHT-2018-19/443, SAWYT-2018-19/309, GS/2018-19/6027, GW 173 and HTWYT/2018-19/36, were grouped in sub cluster C. Another eight genotypes namely, HI 1628, GS/2019-20/3060, 18HTWYT/2019-20/30, EHT-2018-19/403, GS/2019-20/4003, GS/2019-20/7004, EHT-2018-19/406 and GS/2018-19/4049 were grouped in sub cluster D. Remaining two genotypes *viz.*, HTWYT/2019-20/17 and HTWYT/2019-20/39 were included in sub cluster E which showed (0.67) genetic similarity.

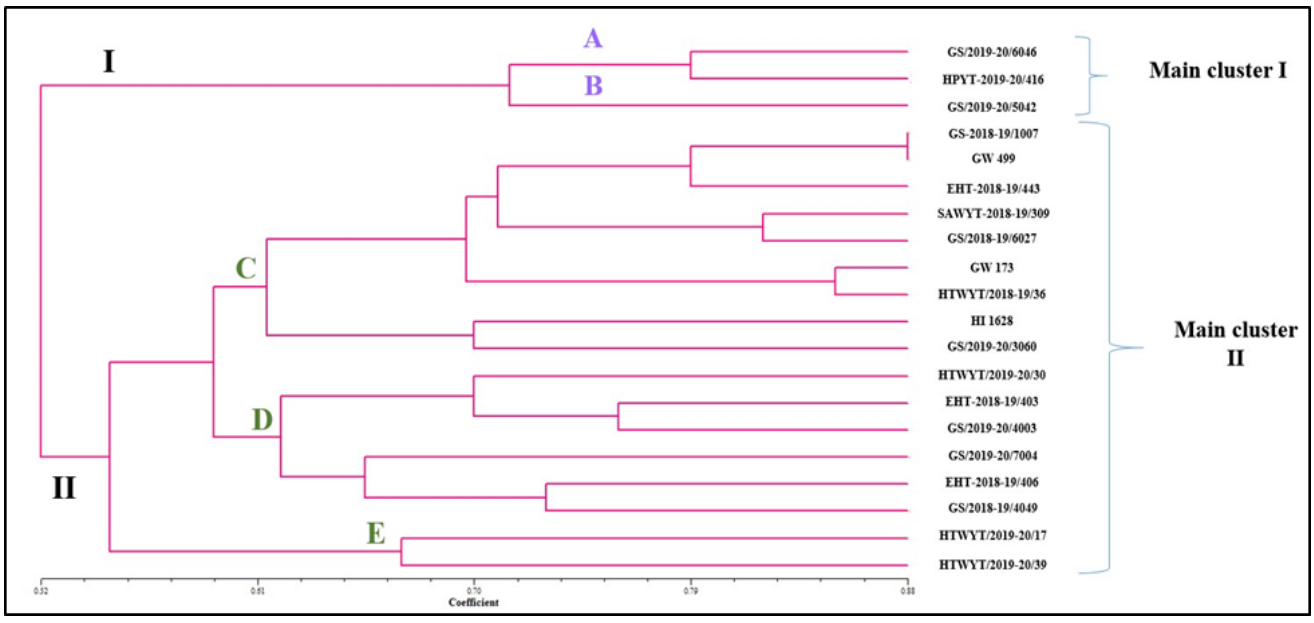

Fig. 3. Dendrogram generated for 20 wheat genotypes using UPGMA cluster analysis based on Jaccard's similarity coefficient using molecular markers markers

Table 5. Jaccard's similarity coefficient matrix for 20 wheat experimental genotypes

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	1.00																			
2	0.46	1.00																		
3	0.49	0.79	1.00																	
4	0.79	0.42	0.58	1.00																
5	0.73	0.55	0.46	0.70	1.00															
6	0.55	0.49	0.64	0.76	0.46	1.00														
7	0.49	0.85	0.64	0.46	0.58	0.64	1.00													
8	0.46	0.52	0.42	0.49	0.55	0.61	0.67	1.00												
9	0.46	0.88	0.79	0.55	0.55	0.61	0.73	0.46	1.00											
10	0.52	0.39	0.61	0.61	0.49	0.67	0.49	0.52	0.46	1.00										
11	0.39	0.52	0.55	0.49	0.36	0.61	0.48	0.46	0.64	0.64	1.00									
12	0.36	0.73	0.81	0.46	0.46	0.58	0.58	0.49	0.73	0.67	0.67	1.00								
13	0.49	0.73	0.64	0.52	0.46	0.64	0.70	0.49	0.73	0.55	0.61	0.64	1.00							
14	0.36	0.61	0.46	0.52	0.64	0.58	0.58	0.67	0.67	0.49	0.61	0.52	0.52	1.00						
15	0.49	0.67	0.58	0.58	0.70	0.58	0.64	0.55	0.79	0.42	0.55	0.64	0.70	0.64	1.00					
16	0.36	0.55	0.64	0.52	0.52	0.70	0.64	0.61	0.67	0.61	0.61	0.70	0.52	0.46	0.64	1.00				
17	0.49	0.73	0.70	0.58	0.52	0.58	0.64	0.42	0.79	0.67	0.73	0.70	0.70	0.64	0.64	0.58	1.00			
18	0.42	0.49	0.52	0.58	0.52	0.70	0.64	0.67	0.55	0.67	0.61	0.64	0.52	0.58	0.52	0.76	0.64	1.00		
19	0.55	0.55	0.58	0.64	0.64	0.58	0.58	0.67	0.55	0.42	0.36	0.58	0.46	0.52	0.70	0.58	0.46	0.64	1.00	
20	0.58	0.70	0.73	0.61	0.61	0.67	0.67	0.52	0.70	0.64	0.64	0.73	0.85	0.49	0.67	0.67	0.73	0.67	0.61	1.00

GS/2019-20/6046
GS-2018-19/1007
SAWYT-2018-19/309
HPYT-2019-20/416
GS/2019-20/5042
HTWYT/2019-20/30
EHT-2018-19/443
8) HTWYT/2019-20/17
9) GW 499
10) GS/2019-20/7004
11) EHT-2018-19/406
12) GS/2018-19/6027
13) GW 173
14) HTWYT/2019-20/39
15) HI 1628
16) EHT-2018-19/403
17) GS/2018-19/4049
18) GS/2019-20/4003
19) GS/2019-20/3060
20) HTWYT/2018-19/36

The genotypes with maximum genetic similarity (0.88) were GS-2018-19/1007 and GW-499, indicating that these genotypes are substantially similar at the genomic level. The minimal genetic similarity (0.36) was noted between GS/2019-20/6046 and GS/2018- 19/6027; GS/2019 -20/6046 and HTWYT/2019-20/39; GS/2019-20/6046 and EHT-2018-19/403; EHT-2018-19/406 and GS/2019-20/5042; GS/2019-20/3060 and EHT-2018-19/406 have been identified as highly diverse at the genomic level. These pairs can be effectively utilized in various applications, such as developing biparental mapping populations and enhancing wheat improvement programs to broaden the genetic diversity among wheat genotypes. GW 499 is recognized as a heat-tolerant variety that has been released in Gujarat and it is a popular choice for late sowing. This study pinpointed two genotypes, GS-2018-19/1007 (0.88) and EHT-2018-19/443 (0.73), which closely resembled the heat-tolerant variety, GW-499. This suggests the presence of genetically related traits associated with heat tolerance in these genotypes. Therefore, regarding molecular grouping, SSR proved to be more effective and dependable in assessing genetic diversity compared to phenotypic diversity.

Conclusion

The current study characterized the polymorphism among wheat genotypes. Furthermore, polymorphisms observed in wheat genotypes by markers located on chromosomes provide opportunities to discover relevant links between these markers and attributes that can contribute to enhanced heat tolerance. Three SSR markers of the present study, i.e., Xwmc603, Xwmc161 and Xgwm577, can be helpful in this regard. Further, using this SSR can increase the speed of wheat breeding, particularly for a heat-stress environment. The highly diverse genotype pairs (GS/2019-20/6046 and GS/2018-19/6027; GS/2019-20/6046 and HTWYT/2019-20/39; GS/2019-20/6046 and EHT-2018-19/403; EHT-2018-19/406 and GS/2019-20/5042; GS/2019-20/3060 and EHT-2018-19/406) can be effectively utilized in heat tolerance breeding programs to develop biparental mapping populations and enhance genetic diversity for improved heat stress resilience in wheat.

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Authors' contributions

CP contributed to the writing, including review and editing, as well as data curation, investigation and was the primary drafter of the manuscript. BK was responsible for design the overall study, set the methodology, project administration and supervision. KM contributed to the methodology, project administration, supervision and data analysis. RV and RK was involved in the study design, performed the statistical analysis and provided resources. VP contributed to the conceptualization, methodology and software analysis. SB, HV, MS and DS assisted in drafting the original manuscript and contributed to its review and editing. All authors reviewed and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: None, The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical issues: None

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