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DEPICTING DNA MAKEUP OF WHEAT GENOTYPES USING SSR MARKERS AND THEIR ASSOCIATION WITH THERMOTOLERANCE

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Abstract

Wheat, a crop highly sensitive to high temperature, faces increased risks due to global warming. This study aimed to analyze DNA to identify wheat genotypes that respond well to heat and to uncover genetic similarities and differences among them. Fifteen wheat genotypes were planted on two dates, November 21 (Irrigated Timely Sowing, ITS) and December 21 (Irrigated Late Sowing, ILS), and analyzed with 13 SSR markers. A dendrogram divided the genotypes into two primary groups, each containing four sub-clusters. The greatest genetic distances were observed between BAW 1290 and BARI gom 28 (0.929), while the smallest genetic distance (0.000) was found between Nadi 2 and BAW 1147. These results suggest that genotypes in both Cluster I and Cluster II (Group A) display moderate tolerance to heat, as indicated by their Heat Susceptibility Index (HSI) for 1000-grain weight (TGW) and grain yield, with HSI values between 0.50 and 1.00, allowing them to perform under heat stress. In contrast, genotypes in Group B, especially those in Cluster III, showed the higher HSI values (1.04-1.23) and significant declines in TGW and yield under heat stress, indicating susceptibility. Similarly, Cluster IV genotypes exhibited heat sensitivity (HSI>1.00). Seven genotypes, specifically BARI gom33, BARI gom32, BARI gom31, BARI gom30, BARI gom29, BARI gom26, and BARI gom25 were identified as suitable for cultivation under heat stress. The findings from this and other studies on heat-tolerant genotypes are valuable for advancing heat-resilient breeding programs based on SSR marker selection.

Keywords: Genetic diversity, Heat stress, SSR marker, Spring wheat.

Introduction

Wheat, the world's staple crop, ranks second in Bangladesh after rice. Global warming threatens wheat production, as rising temperatures impact growth, yield, and food security (Kumer *et al.*, 2022). Abiotic stresses like heat, drought, salinity, and cold disrupt wheat’s genetic integrity and metabolic functions. Climate change is increasing extreme weather events, further exacerbating these challenges (IPCC, 2023; CRP, 2024).

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In Bangladesh, the optimal wheat sowing period is from November 15 to 30, requiring day temperatures ≤25°C and night temperatures ≤16°C for proper growth (Hossain *et al.,* 2023; Alam *et al.,* 2014). However, delayed sowing leads to higher temperatures during grain filling, causing yield losses. Terminal heat stress, particularly above 30°C during reproductive development, significantly reduces yield and quality, affecting pollination and grain filling (Shenoda, *et al.*, 2021). Traditional breeding methods have prioritized yield under ideal conditions, leaving many cultivars susceptible to heat stress. Thus, developing heat-tolerant wheat varieties is crucial (Hossain *et al.*, 2023).

Breeding heat-tolerant wheat involves molecular and biochemical profiling, identifying resistance traits, and leveraging biotechnological advancements (Chaudhary *et al.,* 2020). In 2022-2023, Bangladesh produced 1.16 million metric tons of wheat across 0.31 million hectares, with heat stress threatening productivity (DAE, 2023; BBS, 2023). Wheat's large genome (~17 Gb) complicates breeding, but research has identified key genes, molecular markers, and QTLs associated with heat tolerance (Walkowiak *et al.,* 2020; Chaudhary *et al.,* 2020).

Advances in molecular breeding, including PCR-based markers and SSR markers, have enhanced wheat genetic studies. The Heat Stress Index (HSI) helps evaluate heat-tolerant genotypes based on agronomic performance (Shenoda *et al.,* 2021; Hossain *et al.,* 2023; Bhusal *et al.*, 2017). Bangladesh Wheat and Maize Research Institute (BWMRI) has released 38 wheat varieties, some potentially heat-tolerant, though their precise temperature tolerance remains unclear (BWMRI, 2024). This study aims to characterize these varieties, identify heat-responsive genotypes through genetic analysis, and enhance wheat's resilience to climate change.

Materials and Methods

Plant materials

A total of fifteen bread wheat genotypes (*Triticum aestivum* L.)- comprising thirteen established varieties and two advanced lines- were selected to assess their molecular diversity in terms of tolerance to terminal heat stress. These genotypes were evaluated using thirteen Simple Sequence Repeat (SSR) markers, which are highly effective in identifying genetic variability and molecular characteristics linked to heat tolerance traits. Each genotype was sourced from the Bangladesh Wheat and Maize Agricultural Research Institute (BWMRI), located in Nashipur, Dinajpur, Bangladesh. BWMRI's focus on the development and selection of heat-tolerant wheat varieties made it an ideal source for obtaining seeds of the genotypes used in this study. Table 1 provides a detailed summary of each genotype's pedigree, along with their specific attributes, such as growth habits, phenological traits, and any previously documented heat tolerance traits, thus setting the foundation for understanding their potential in high-temperature environments.

Table 1. List of fifteen wheat genotypes with their pedigrees

Variety	Pedigree	Year of release	Life cycle (d)	Salient features
BARI gom25	ZSH 12/HLB 19//2*NL297	2010	102-110	• This variety tolerates heat stress, and salinity 8.0-10 dS/m salinity at the seedling stage • Grain yield (3.9-5.2 t ha^{-1}) • It is resistant to leaf rust (LR) and *Bipolaris* leaf blight (BpLB) diseases
BARI gom26	ICTAL 123/3/RAWAL 87//VEE/HD 2285 BD(JO)9585-0JO-3JE-0JE-0JE-HRDI-RC5DI	2010	104-110	• Heat tolerance, and appropriate for late sowing • It is high yielding variety (4.5-5.2 t ha^{-1}) • This variety displays strong resistance to leaf rust (LR), tolerance to the aggressive Ug99 race of stem rust, and moderate resistance to brown leaf spot (BpLB). These traits enhance its value for cultivation in disease-prone areas, increasing its potential for higher yields and improved resilience in challenging growing conditions.
BARI gom27	WAXWING*2/VIVISTI CGSS01BOOO56T-099Y-099 M-099 M-099Y-099 M-14Y-0B	2012	105-110	• It is high yielding variety (3.6-5.5 t ha^{-1}) • Having resistant to LR and stem rust (Ug99 race), with moderate resistance to BpLB.
BARI gom28	CHIL/2*STAR/4/BOW/CROW//BUC/PVN/3/2*VEE#10 CMSS95Y00624S-0100Y-0200 M-17Y-010 M-5Y-0 M	2012	102-108	• Having early maturing and heat tolerant attributes. • Gives good yield in the late seeding condition. • Grain yield (4.5-5.0 t ha^{-1}) • Having Resistant and moderately resistant to LR and BpLB, respectively
BARI gom29	SOURAV/7/KLAT/SOREN//PSN/3/BOW/4/VEE#5. 10/5/CNO 67/MFD//MON/3/SERI/6/NL297 BD(DI)112S-0DI-030DI-030DI-030DI-9DI	2014	105-110	• Having moderately tolerant attribute to high temperature • Grain yield (4.5-5.0 t ha^{-1}) • Having moderately resistant to BpLB. It is also resistant to LR and stem rust
BARI gom30	BAW 677/Bijoy BD(JA)1365S-0DI-15DI-3DI-HR12R3DI	2014	100-105	• This is a very high-yielding variety. Grain yield: 4.5-5.5 t ha^{-1} • Exhibits resistance to leaf rust and moderate resistance to brown leaf spot (BpLB) • It has heat-tolerant attributes and • very suitable for late sowing
BARI gom31	KAL/BB/YD/3/PASTOR CMSS99M00981S-0P0M-040SY-040 M-040SY-16 M-0ZTY-0 M	2017	104-109	• Early maturing, heat tolerance • Resistant to LR and tolerant to spot blotch • Yield:4.5-5.0 t ha^{-1}

Variety	Pedigree	Year of release	Life cycle (d)	Salient features
BARI gom32	SHATABDI/GOURAB BD(DI)1686S-0DI-1DI-0DI-0DI-3DI	2017	95-105	• This variety possesses early maturing, heat tolerant, and short stature attributes • Generally, shows resistant to LR and tolerant to spot blotch • It tolerates newly found wheat blast disease (only 10-12% infection observed in Jashore region) • Grain yield: 4.5-5.5 t ha^{-1}
BARI gom33	KACHU/SOLALA	2018	110-115	• Culm robust, succulent, firm, erect, and found lodging under high wind flow • Leave and stem deep green from seedling to anthesis stages. So, farmers prefer it too much • Resistant to blast disease, LR, and tolerant to terminal heat stress • Grain zinc enriched (50-55 ppm)
BWMRI gom1	BARI gom21 (Shatabdi)/BARI gom24	2019	100-104	• Short stature, tolerant to lodging. • Having the shortest life cycle of all released varieties and early maturing, escaping terminal heat stress • Tolerant to blast and rust diseases
BWMRI gom2	BARI gom26/BARI gom 25	2021	108-115	• Grain in amber color • Panicle tall in length, 45-48 grains/panicle, 1000-grain weight 45-50 g • This variety shows resistant attributes to LR and is tolerant to high-temperature • Grain yield: 4.5-5.8 t ha^{-1}
BWMRI gom3	Borlaug 100 ROELFS-F-2007/4/BOBWHITE/NEELKANT//CATBIRD/3/CATBIRD/5/FRET-2/TUKURU//FRET-2	2021	108-114	• Dwarf sized and almost no lodging attribute • Resistant to LR, BpLB, and blast disease • Yield: 4.0-4.5 t ha^{-1}
BAW 1290	BARI gom21/BL 3503	-	-	-
BAW 1147	OASIS/3*ANGRA//708	-	-	-
Nadi 2	-	-	-	-

Table 2. Characteristics of 13 linked SSR markers used in the characterization

SL No.	Marker	QTL for	Primers sequence Reverse (5'- 3')	Primers sequence Forward (5'- 3')	Chromosomal location	Annealing temp (°C)
1	gwm291	Leaf Curl	AATGGTATCTATTCCGACCCG	CATCCCTAGGCCACTCTGC	5A	60
2	Gwm325	HSI grain filling duration HSI kernel weight	TTTTTACGCGTCAACGACG	TTTCTTCTGTCGTTCTCTTCCC	6D	60
3	Xgwm294	HIS single kernel weight of the main spike	GCAGAGTGATCAATGCCAGA	GGATTGGAGTTAAGAGAGAACCG	2A	55
4	Gwm268	HSI kernel weight	TTATGTGATTGCGTACGTACCC	AGGGGATATGTTGTCACTCCA	1B	55
5	Xwmc407	Grain-filling duration	CATATTTCCAAATCCCCAACTC	GGTAATTCTAGGCTGACATATGCTC	2A	61
6	Xcfa2129	HIS single kernel weight of the main spike	ATCGCTCACTCACTATCGGG	GTTGCACGACCTACAAAGCA	1A, 1B, 1D	60
7	gwm11	Grain-filling duration	GTGAATTGTGTCTTGTATGCTTCC	GGATAGTCAGACAATTCTTGTG	1A, 1B	50
8	Xcfd43	Grain-filling duration	CCAAAAACATGGTTAAAGGGG	AACAAAAGTCGGTGCAGTCC	2D	60
9	Xgwm356	HSI single kernel weight of the main spike	CCAATCAGCCTGCAACAAC	AGCGTTCTTGGGAATTAGAGA	2A, 6A, 7A	55
10	Xbarc137	Waxiness	CCAGCCCCTCTACACATTTT	GGCCCATTTCCCACTTTCCA	1B	52
11	Gwm484	Waxiness	AGTTCCGGTCATGGCTAGG	ACATCGCTCTTCACAAACCC	2D	55
12	Gwm293	Grain-filling duration	TCGCCATCACTCGTTCAAG	TACTGGTTCACATTGGTGCG	5A	55
13	WMC527	HIS kernel weight of the main spike	GCTACAGAAAACCGGAGCCTAT	ACCCAAGATTGGTGGCAGAA	3A, 3B	61

Experimental site and sowing date

During the Rabi season (15 Oct to 15 Mar), the genotypes were evaluated at the BWMRI Regional Station (RS) research farm, Joydebpur, Gazipur, which is located in the agro-ecological zone 28 (Madhupur Tract) (FAO/UNDP, 1988). This region is distinguished by intricate relief and soils that have grown on the Madhupur Clay. The field experiments were conducted followed by RCBD with three replications. The plot size was 4 feet × 3 feet. The sub-plots were fertilized @ 100-27-50-20-1-4.5- 5000kg ha^{-1} as N-P-K-S-B-Zn-Cowdung as the source of urea, TSP, MoP, Gypsum, Boric acid, and Zinc sulphate, respectively. TSP, MoP, Gypsum, Boric acid, Zinc sulphate, Cowdung and two-third of urea were applied as basal dose at last ploughed. Seeds were treated with Provax 200 WP@3g kg^{-1} seed, containing Carboxin and Thiram. Seeds @100 kg ha^{-1} were sown continuously in line. Seeds were sown on two dates viz. Nov 21 (ITS) and Dec 21 (ILS). The cultural practices were performed as the recommended guidelines of the BWMRI (2024). Laboratory experiments took place at the Biotechnology Division of the Bangladesh Agricultural Research Institute and BWMRI Regional Station Molecular Laboratory in Joydebpur, Gazipur.

Extraction of DNA and SSR analysis

Genomic DNA was isolated from fresh leaves of fifteen wheat genotypes using a modified CTAB method. From various chromosome sites, thirteen SSR markers (gwm291, Gwm325, Xgwm294, Gwm268, Xwmc407, Xcfa2129, gwm11, Xcfd43, Xgwm356, Xbarc137, Gwm484, Gwm293, WMC527) were chosen. Fresh leaves were grounded in a mortar, then placed in a 2 ml tube, 400 µl chloroform was added, and the mixture was heated for an hour at 65°C. The supernatant was moved to a fresh tube after centrifuging the sample for ten minutes at 4ºC at 12000 rpm. Following the addition and mixing of isopropyl alcohol, the samples were kept at -20°C for two hours. The DNA pellet was centrifuged one more, cleaned with 75% ethanol, allowed to air dry for a full day, and dissolved in 100µl of 1X TE buffer. Thirteen primer pairs were utilized for SSR analysis, and a spectrophotometer was used to verify the quality and concentration of the DNA. PCR parameters were adhered to Röder et al. (1998).

Heat susceptibility index (HSI)

The Heat Susceptibility Index (HSI) was used to assess the impact of heat stress on thousand-grain weight (TGW) and grain yield. The HSI was calculated using the formula provided by Paliwal et al. (2012).

$$HSI\ of\ X = [(1 - X_{heat\ stress}/X_{control})/D]$$

where

X represents TGW and grain yield

$X_{heat\ stress}$ represents phenotypic values of individual genotypes for TGW and grain yield under late sowing.

Control represents phenotypic values of individual genotypes for TGW and grain yield under normal sowing conditions (control conditions).

$$D_{(stress\ intensity)} = (1 - Y_{heat\ stress}/Y_{control})$$

$$Y_{heat\ stress} = Mean\ of\ X_{heat\ stress}\ of\ all\ genotypes$$

$$Y_{control} = Mean\ of\ X_{control}\ of\ all\ genotypes$$

Statistical analysis

A bivariate 1-0 data matrix was created from scorable loci, assigning one locus to each band. Genetic distances between genotypes were quantified using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) based on shared alleles. The values were designed to estimate genetic diversity using PowerMarker software (Version 3.25), dendrograms, and polymorphism information content (PIC) (Botstein et al., 1980**)**.

Results

The thirteen SSR primers were used in this study (Table 2). The information provided in Table 2 included the primer sequences, their lengths, and any relevant details regarding their application. The primers' allele counts and sizes were presented in Table 3. Screening 15 genotypes with 13 SSR markers yielded 51 polymorphic alleles, averaging 3.92 per locus. The primers detected 2.0 to 8.0 alleles per genotype. Gwm293, with eight alleles, was the most polymorphic microsatellite marker, followed by Xgwm356 (having seven alleles) (Table 3; Fig. 1). Xcfd43 had the lowest alleles (two). The markers exhibited Polymorphism Information Content (PIC) values that varied significantly, ranging from a low of 0.33 for the marker Xwmc407 to a high of 0.83 for the marker Xgwm356. The average PIC value across all markers was calculated to be 0.57, indicating a moderate level of polymorphism within the selected markers. These values were presented in Table 3 and illustrated in Figure 1.

Table 3. Allele numbers, sizes, and PIC values for fifteen wheat genotypes for thirteen SSR markers.

Marker	Allele No	Allele size and range	Difference (bp)	Major Allele Frequency	Gene Diversity	Heterozygosity	PIC
gwm291	3	150-160	10	0.46	0.64	0.00	0.57
Gwm325	3	150-160	10	0.38	0.66	0.00	0.58
Xgwm294	4	50-120	70	0.50	0.65	1.00	0.59
Gwm268	3	180-285	105	0.67	0.48	0.11	0.40
Xwmc407	2	140-145	5	0.71	0.41	0.00	0.33
Xcfa2129	4	120-190	70	0.47	0.66	1.00	0.59
gwm11	3	200-210	10	0.71	0.44	0.00	0.39
Xcfd43	2	160-165	5	0.50	0.50	0.00	0.38
Xgwm356	7	185-230	45	0.20	0.57	0.80	0.83
Xbarc137	4	245-260	15	0.44	0.67	0.00	0.61
Gwm484	4	90-190	100	0.36	0.71	0.92	0.66
Gwm293	8	105-190	85	0.23	0.84	1.00	0.82
WMC527	4	345-450	105	0.40	0.70	0.00	0.65
Mean	3.92 (total 51)	-	-	0.46	0.63	0.37	0.57
Range	2.0-8.0	-	2.00 - 105	0.20 - 0.71	0.49-0.85	0.00 - 1.00	0.33-0.83

The primers Xgwm294, Xcfa2129, and Gwm293 exhibited the highest heterozygosity (He) values of 1.00, while the lowest values ranged from 0.33 for Xwmc407 to 0.83 for Xgwm356, resulting in an average PIC value of 0.57. Notably, the primers gwm291, gwm325, Xwmc407, gwm11, Xcfd43, and Xbarc137 all recorded He values of 0.0. Heterozygosity was widely used to assess genetic variation within populations (Table 3). For the microsatellites included in this investigation, PIC exhibited a strong positive connection with the number of alleles (Table 4). Table 4 displayed the highest amount of genetic diversity (0.85) found at locus Xgwm356 and the lowest level of genetic diversity (0.44) found at locus gwm11. Gene diversity was higher in markers that detected more significant alleles than in markers that detected fewer alleles. A dendrogram was created to illustrate the genetic distances between common alleles derived from 15 genotypes and 51 alleles (Fig. 2).

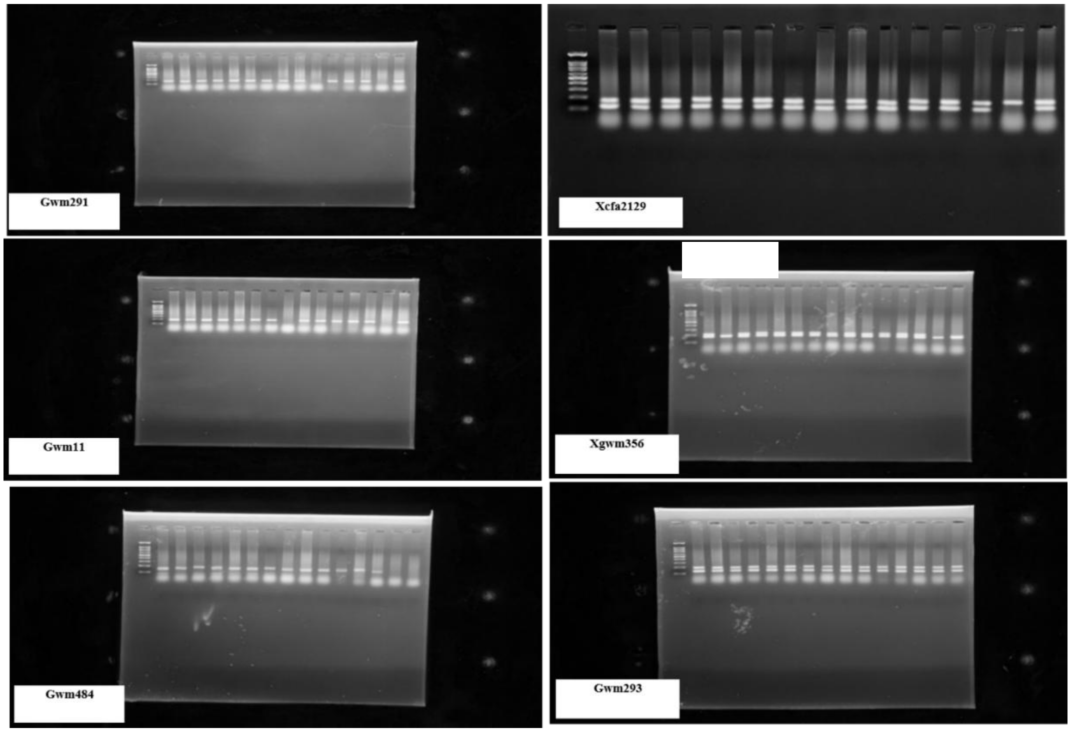

Fig. 1. Profiles of wheat genotypes were created using SSR markers with the primers Gwm291, Xcfa2129, Gwm11, Xgwm356, Gwm484, and Gwm2. In the picture (Left to right), the genotypes showed the bands: 1. BARI gom25, 2. BARI gom26, 3. BARI gom27, 4. BARI gom28, 5. BARI gom29, 6. BARI gom30, 7. BARI gom31, 8. BARI gom32, 9. BARI gom33, 10. BWMRI gom1, 11. BWMRI gom2, 12. BWMRI gom3, 13. BAW 1290, 14. BAW 1147, 15. NADI 2(CB -83), respectively.

Table 4. Genetic distance of fifteen wheat genotypes based on thirteen SSR markers

	BARI gom25	BARI gom26	BARI gom27	BARI gom28	BARI gom 29	BARI gom 30	BARI gom 31	BARI gom 32	BARI gom33	BAW 1147	BAW 1290	Nadi 2	BWMRI gom1	BWMRI go 2	BWMRI gom 3
BARI gom25	0.000														
BARI gom26	0.150	0.000													
BARI gom27	0.545	0.500	0.000												
BARI gom28	0.583	0.500	0.458	0.000											
BARI gom29	0.583	0.600	0.591	0.250	0.000										
BARI gom30	0.667	0.700	0.591	0.333	0.083	0.000									
BARI gom31	0.708	0.650	0.727	0.542	0.292	0.292	0.000								
BARI gom32	0.714	0.786	0.500	0.714	0.571	0.571	0.571	0.000							
BARI gom33	0.714	0.786	0.571	0.786	0.500	0.500	0.429	0.143	0.000						
BAW 1147	0.750	0.750	0.667	0.875	0.875	0.875	0.875	0.667	0.833	0.000					
BAW 1290	0.714	0.714	0.714	0.929	0.786	0.786	0.786	0.500	0.583	0.333	0.000				
Nadi 2	0.800	0.800	0.800	0.900	0.700	0.700	0.700	0.400	0.500	0.000	0.200	0.000			
BWMRI gom1	0.714	0.786	0.500	0.786	0.500	0.500	0.286	0.333	0.167	0.875	0.700	0.625	0.000		
BWMRI gom2	0.700	0.813	0.778	1.000	0.800	0.800	0.600	0.417	0.333	0.875	0.500	0.500	0.167	0.000	
BWMRI gom3	0.800	0.889	0.667	0.900	0.700	0.700	0.600	0.417	0.333	0.625	0.214	0.300	0.333	0.333	0.000

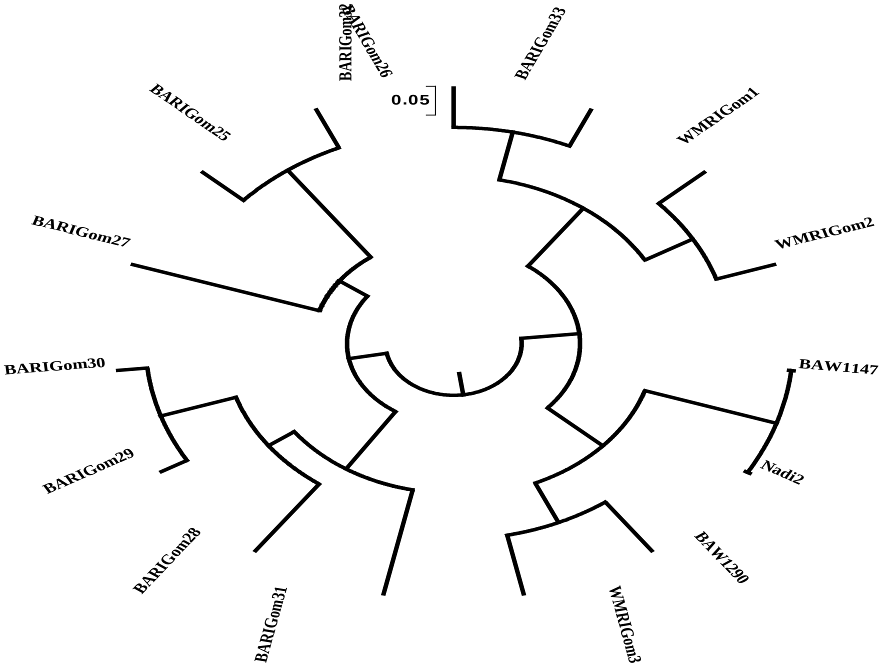

Fig. 2. The dendrogram, generated through UPGMA analysis, illustrates the genetic relationships among fifteen wheat genotypes, with the names of the genotypes listed at the ends of the branches.

Fifteen genotypes could easily be identified as heat tolerance or susceptible. The UPGMA cluster tree analysis drew the dendrogram with those fifteen genotypes using a distance coefficient of 0.050 (Fig. 2). Values ranging from 0.000 to 0.929 were obtained from the combined data for the 13 primers in pairwise comparisons of shared alleles and genetic distances across the variations (Table 4). Tables 5 and 6 display the mean TGW and grain yield HSI values, respectively. The analysis examined TGW and grain yield under ILS and ITS conditions, along with the mean percentage change in these parameters between the two conditions for each cluster member. The HSI, TGW, and grain yield under stress conditions, along with the percent decrease in TGW and grain yield from ILS to ITS, were key parameters that differentiated the two major groups identified in the cluster analysis as indicators of heat tolerance under field conditions (Tables 5, 6).

Table 5. Summary of wheat genotype clusters according to thousand grain weight (TGW)

Cluster	Genotypes	HSI	TGW		%TGW decrease	HSI	TGW		%TGW decrease
			ITS	ILS			ITS	ILS	
Group A									
Cluster I	BARI gom25	0.887	49.00	43.87	10.47	0.887	49.00	43.87	10.47
	BARI gom26	0.885	48.05	43.03	10.45				
	BARI gom27	0.870	42.85	38.45	10.27				
Cluster II	BARI gom28	0.772	45.00	40.90	9.11	0.772	45.00	40.90	9.11
	BARI gom29	0.892	42.35	37.89	10.53				
	BARI gom30	0.823	46.80	42.25	9.72				
	BARI gom31	0.909	42.75	38.16	10.74				
Group B									
Cluster III	BWMRI gom3	1.038	43.15	37.86	12.26	1.038	43.15	37.86	12.26
	BAW 1290	1.181	44.30	38.12	13.95				
	BAW 1147	1.218	45.05	38.57	14.38				
	Nadi 2	1.106	43.55	37.86	13.07				
Cluster IV	BARI gom32	1.169	48.55	41.85	13.80	1.169	48.55	41.85	13.80
	BARI gom33	1.061	48.85	42.73	12.53				
	BWMRI gom1	1.129	48.85	42.34	13.33				
	BWMRI gom2	1.043	49.30	43.23	12.31				

Table 6. Summary of wheat genotype clusters according to grain yield

Cluster	Genotypes	HSI	Yield		% Yield decrease	HSI	Yield		% Yield decrease
			ITS	ILS			ITS	ILS	
Group A									
Cluster I	BARI gom25	0.903	2.68	2.31	13.81	0.903	2.68	2.31	13.81
	BARI gom26	0.964	2.17	1.85	14.75				
	BARI gom27	0.960	2.52	2.15	14.68				
Cluster II	BARI gom28	0.742	2.38	2.11	11.34	0.742	2.38	2.11	11.34
	BARI gom29	0.833	2.59	2.26	12.74				
	BARI gom30	0.756	2.68	2.37	11.57				
	BARI gom31	0.930	2.67	2.29	14.23				
Group B									
Cluster III	BWMRI gom3	1.128	1.97	1.63	17.26	1.128	1.97	1.63	17.26
	BAW 1290	1.189	2.64	2.16	18.18				
	BAW 1147	1.253	2.66	2.15	19.17				
	Nadi 2	1.135	2.65	2.19	17.36				
Cluster IV	BARI gom32	1.050	2.49	2.09	16.06	1.050	2.49	2.09	16.06
	BARI gom33	1.004	2.54	2.15	15.35				
	BWMRI gom1	1.102	2.61	2.17	16.86				
	BWMRI gom2	1.056	2.60	2.18	16.15				

The HSI was evaluated for TGW and grain yield to identify heat-tolerant and heat-susceptible genotypes. Grain yield ranged from 0.74 to 1.25, while the HSI for TGW varied from 0.77 to 1.23. Data from this assessment revealed heat-tolerant genotypes, with heat-stress tolerance correlated with a low HSI (HSI<1) (Table 5). Genotypes were categorized according to their HSI values, which serve as an important indicator of their tolerance to heat stress. The classification is as follows: those with high heat tolerance were defined as having an HSI value of less than 0.50, indicating a strong ability to maintain performance under elevated temperatures. Genotypes that exhibited moderate heat tolerance were identified with HSI values ranging from 0.50 to 1.00, suggesting a more variable response to heat stress but still capable of reasonable yield performance. In contrast, genotypes classified as heat-sensitive displayed HSI values greater than 1.00, indicating a significant decline in performance under heat-stress conditions. This classification system is based on the framework established by Khanna-Chopra and Viswanathan (1999). Table 5 summarizes the mean HSI values for TGW under ITS and ILS conditions, the mean percentage change in TGW and grain yield between ILS and ITS, and the corresponding values for each cluster member. The three key parameters that differentiated the two major groups identified in the cluster analysis as indicators of heat tolerance under field conditions were the HSI, TGW, and grain yield under stress, along with the percent decrease in TGW and grain yield from ILS to ITS (Table 5).

Three genotypes, BARI gom25, BARI gom26, and BARI gom27, comprised Cluster I. Their respective HSI values for TGW and grain yield were more significant, falling between 0.885-0.887 and 0.903-0.964, respectively (Tables 5 and 6). The heat tolerance of these genotypes was moderate (HSI 0.50-1.00). Under ILS, the TGW (g) and grain yield/plot (kg) varied from 38.5 to 43.8 and 1.85 to 2.31, respectively. When comparing the grain yield under the ILS to the ITS, these genotypes' TGW dropped from 10.3 to 10.5%, and their grain yield dropped from 13.7 to 14.8% (Tables 5 and 6). The TGW and grain yield cluster means, and the corresponding declines in TGW and grain yield were 0.887, 0.903, 10.5%, and 13.8%, respectively. These genotypes closely matched group A's cluster II members (Tables 5 and 6). Cluster II was composed of four distinct genotypes: BARI gom28, BARI gom29, BARI gom30, and BARI gom31. These genotypes were grouped based on their genetic similarities and shared characteristics, which likely reflect their responses to environmental stresses such as heat. The inclusion of these specific genotypes in Cluster II indicates their potential for comparable performance traits, making them valuable for further studies and breeding programs aimed at enhancing heat tolerance in wheat. Their classification within this cluster allows researchers to explore the genetic diversity and agronomic traits associated with each genotype, facilitating the identification of candidates for cultivation in regions affected by elevated temperatures.

The ranges of the HSIs for TGW and grain yield, as well as the decreases in TGW and yield from ITS to those under ILS, were 0.772-0.903, 0.742-0.930, 9.11-10.7%, and 11.3-14.2%, respectively. These findings open up new avenues for understanding and improving heat tolerance in wheat. The losses in TGW and grain yield, as well as the cluster means of the HSI for these variables, were 0.772, 0.742, 9.11%, and 11.3%, respectively. Additionally, these genotypes have a moderate tolerance to heat (HSI 0.50-1.00). Eight genotypes, separated into two clusters (III and IV), comprised Group B. Four genotypes made up Cluster III were BWMRI gom3, BAW 1290, BAW 1147, and Nadi 2. For this cluster, the mean HSIs were 1.04-1.23, 1.135-1.253, 12.3-14.4%, and 17.3-19.2% for TGW, grain yield, and the proportional decreases in TGW and grain yield under ILS compared to ITS, respectively. According to the HSI, TGW and grain yield's cluster means and the decreased percentages of TGW and grain yield were 1.04, 1.13, 12.3%, and 17.3%, respectively. There was heat susceptibility in these genotypes (HSI>1.00). Cluster IV was made up of four genotypes: BARI gom32, BARI gom33, BWMRI gom1, and BWMRI gom2 For this cluster, the mean HSIs for TGW and grain yield were 1.06-1.17 and 1.004-1.1.102, respectively; the relative decreases in TGW and grain yield under ILS compared to ITS were 12.5-13.8% and 15.4-16.9%. Additionally, some genotypes showed heat sensitivity (HSI>1.00). The losses in TGW and grain yield, as well as the cluster means of the HSI for these variables, were 1.17, 1.05, 13.8%, and 16.1%, respectively.

Discussion

Genetic similarity information ensures long-term productivity gains during breeding operations, which prevents elite germplasm from becoming homogenous. Numerous unique genes are probably present in cultivars with different DNA profiles. Both phenotypic and molecular data made practical assessments of genetic variation and heat-tolerant genotypes possible. SSR markers provided helpful information for DNA fingerprinting and genetic diversity estimation (Gupta *et al.*, 2022). They generated distinct bands for heat-tolerant genotypes, suggesting their potential use in enhancing heat tolerance. By integrating phenotypic and molecular data, wheat varieties with improved yields under abiotic stress could be developed (Haliloglu et al., 2022).

The 13 SSR markers produced 51 polymorphic bands, yielding an average PIC of 0.57. The number of bands per marker varied from 2 for Xcfd43 and Xwmc407 to 7 for Xgwm356, with an overall average of 3.92 bands. PIC values varied between 0.38 for the marker Xcfd43 and 0.83 for the marker Xgwm356, highlighting the informative nature of certain SSR markers. This variation in PIC values reflects the different levels of genetic diversity captured by these markers, with higher values, indicating a greater ability to distinguish between genotypes. Consequently, markers like Xgwm356 could provide more detailed insights into genetic relationships and variations among the wheat genotypes, making them valuable tools for studies aimed at understanding and enhancing traits such as heat tolerance. This outcome was similar to what Sharma *et al.* (2017) found. Variations in allele frequency may cause PIC value differences. Polymorphic bands revealed changes between genotypes, allowing researchers to analyze systematic links (Haliloglu *et al.*, 2022). Eleven SSR markers produced distinct bands for heat-tolerant genotypes; these markers may be utilized to indicate heat tolerance, but further testing in a range of populations is required. Table 3 indicates that distinct chromosomes host SSR markers associated with heat tolerance. The SSR markers utilized in this study demonstrated low heterozygosity, with an average value of 0.37 among the examined wheat genotypes, indicating limited genetic variation. Thirteen loci with PIC values exceeding 0.50 were identified as informative, as a PIC above 0.5 signifies high diversity, while a PIC below 0.25 indicates low diversity (Nagy *et al.*, 2012; Ramadugu et al., 2015). The mean PIC for the SSR markers was 0.57, with values ranging from 0.33 to 0.83 (Table 3). Thus, most primers provided valuable information, demonstrating that SSRs are effective markers for selecting terminal heat stress tolerance in molecular plant breeding. Mourad *et al.* (2020) suggested that evaluating the number of alleles at each locus alongside their PIC values is essential for objectively assessing genetic diversity in genotyping collections.

Each of the four clusters exhibited distinct traits. Cluster IV had the highest mean HSI value (>1.00) and the largest decrease in TGW under ILS (sown on December 21) compared to ITS (sown on November 21) (Table 5). In contrast, cluster III showed the highest mean HSI value and the most significant decline in grain yield under ILS relative to ITS (Table 6). Notably, BARI gom25 and BARI gom30 demonstrated greater genetic potential for yield under ILS, outperforming other genotypes in group A. BAW 1147 was categorized as heat-sensitive due to its higher HSI for grain yield (Table 6). According to Table 6, the genotypes in group A-BARI gom25, BARI gom26, BARI gom27, BARI gom28, BARI gom29, BARI gom30, and BARI gom31 were found to be well-suited for ILS. Similar findings were reported by Alam *et al.* (2014). Although few discrepancies were observed, most morphological data confirmed the molecular findings. For instance, Nadi 2 in group B showed a higher HSI (1.135) and a more notable decrease in mean grain production (17.4%) under ILS compared to ITS (Table 6). It should not be categorized as heat-sensitive because it had the highest grain yield in the ILS despite being in the heat-sensitive group. In contrast, BARI gom26 in group A had a higher HSI value (0.97) but the most significant drop in mean grain output (14.8%) under ILS and the lowest grain yield within the heat-tolerant group. This was comparable to the grain yield of WMRI gom3 in group B, indicating that it should not be categorized as a terminal heat stress-tolerant group. These variations could be attributed to the regional diversity of heat stress, which affects plants differently depending on length and timing. Heat stress significantly affects yield, with genotype-environment interactions playing a vital role in yield expression. This field study, reflecting noticeable weather variations, aligns with the findings of Haliloglu *et al.* (2022). High temperatures above the optimal range (21.3±1.27°C) during the grain-filling stage adversely impact wheat output, underscoring the need for developing heat-resistant wheat varieties. It is essential to identify or develop genotypes capable of withstanding terminal heat stress or maturing early with minimal yield losses (Shenoda *et al.*, 2021). The molecular and genetic techniques utilized in this study, such as marker-assisted breeding and DNA polymorphism characterization, enabled the identification of both heat-tolerant and heat-sensitive wheat genotypes. The effective distinction of these genotypes through SSR markers contributes to the development of future heat-tolerant wheat varieties and supports the establishment of intellectual property.

Conclusion

SSR markers provided valuable information for DNA fingerprinting and genetic diversity estimates, producing distinct bands for heat-tolerant genotypes. The study assessed fifteen wheat genotypes, creating a dendrogram with a distance coefficient of 0.050 and dividing them into two main categories. The greatest genetic distances were observed between BAW 1290 and BARI gom28 (0.929), and the lowest genetic distance of 0.000 was noted between Nadi 2 and BAW 1147. Group A, which included seven genotypes from Clusters I and II BARI gom25, BARI gom26, BARI gom27, BARI gom28, BARI gom29, BARI gom30, and BARI gom31, was identified as suitable for cultivation under heat-stressed conditions. This study focused on analyzing DNA to identify both heat-tolerant and heat-sensitive wheat genotypes, providing valuable insights for future molecular breeding programs aimed at enhancing thermotolerance.

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Authors' contribution

MNA and AR conceptualized and designed the experiments. MFA conducted the trials. MNA supervised the projects. The manuscript was written by MNA and MFA. It was revised and edited by AR. All authors approved the final version.

Conflict of Interest

All authors affirm that there are no conflicts of interest regarding this research paper.

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