Preprints.org

Article

Depicting of DNA Makeup of Spring Bread Wheat Genotypes Using SSR Markers and Their Association with Heat Tolerance

Md. Forhad Amin , Mohammad Nur Alam [*] , Golam Faruq , Md. Mustafa Khan , Md. Mahbubur Rahman

Article

Depicting of DNA Makeup of Spring Bread Wheat Genotypes Using SSR Markers and Their Association with Heat Tolerance

Md. Forhad Amin [1], **Mohammad Nur Alam** [2,*], **Golam Faruq** [1,2,*], **Md. Mustafa Khan,** [1] **and Md. Mahbubur Rahman** [1]

[1]   Regional Station, Bangladesh Wheat and Maize Research Institute, Joydebpur, Gazipur, 1701, Bangladesh
[2]   Bangladesh Wheat and Maize Research Institute, Nashipur, Dinajpur, 5200, Bangladesh
*   Correspondence: nur.alam@bwmri.gov.bd (M.N.A.); dg.bwmri@bwmri.gov.bd (G.F.);
    Tel.: +880-1724-921904 (M.N.A.)

Abstract: Wheat is threatened by global warming. This study aimed to characterize based on DNA profile and identify heat-tolerant wheat genotypes. Fifteen wheat genotypes were sown in Nov 21 and Dec 21 and evaluated within 13 SSR markers. A dendrogram classified the genotypes into two main groups. The highest genetic distances (0.93) were drawn between BAW 1290 and BARI Gom 28, and the lowest one between Nadi 2 and BAW 1147. Heat susceptibility index (HSI) values for 1000-grain weight (TGW) and grain yield indicated moderate heat tolerance in Cluster I genotypes (HSI 0.50-1.00), closely matching Group A's Cluster II members. In Group B, Cluster III genotypes had higher HSI (1.04-1.23) and significant reductions in TGW and grain yield under heat stress, indicating heat susceptibility. The varieties BARI Gom 25, 26, 27, 28, 29, and 30 were identified as heat-stress tolerant. This research provides insights for future heat stress-molecular breeding programs.

Keywords: bread wheat; fingerprinting; genetic diversity; heat tolerance; SSR marker

Introduction

Global warming seriously threatens wheat crops worldwide because wheat is a cold-loving plant. The temperature increase directly impacts crop production activities since various climatic events have increased or altered them and threaten global food security (Kumer et al. 2022). The exposure of crops to abiotic stresses, such as heat stress, drought, salinity, cold, etc., affects the development and growth and hinders the genetic makeup, and metabolic and physiological activities of plants (Alam et al. 2018a, 2021; dos Santos et al. 2022). Climate change is expected to increase the frequency of extreme weather events such as floods, droughts, high temperatures, etc. (Figure 1) (IPCC 2023; CRP 2024). Heat is a primary abiotic stressor that reduces crop output and degrades its quality. Heat and climate change hurt wheat production (Sánchez-Bermúdez et al. 2022).

According to the IPCC (2023), human activities have caused global surface air temperature to rise from 0.8 to 1.3°C (avg. 1.07°C) between 1850 and 1900 and 2010-2019. The passage has discussed the estimated contributions of various factors to global surface temperature rises, such as well-mixed greenhouse gases, other human drivers, primarily aerosols, natural drivers (solar and volcanic), and internal variability, which range from 1.0°C to 2.0°C, 0.0°C to 0.8°C, -0.1°C to 0.1°C, and -0.2°C to 0.2°C, respectively. The worldwide surface air temperature increased by 1.09°C (range: 0.95 to 1.20°C) between 2011 and 2020 compared to 1850-1900, with more significant increases over land (1.59°C (range: 1.34 to 1.83°C) than over the ocean (0.88°C) (range: 0.68 to 1.01 °C). IPCC (2018) forecasts show a further increase in annual mean temperatures of 1.0°C by 2030, 1.4°C by 2050, and 2.4°C by 2100. The winter season (Dec, Jan, and Feb) is on a trajectory of significant temperature increases, with a rise of 1.1°C by 2030, 1.6°C by 2050, and a staggering 2.7°C by 2100. This rapid change is a cause for immediate concern. From 1949 to 2013, the annual mean, min, and max temperatures increased by 0.13°C each decade. Temperatures increased by 0.18°C per decade (post-monsoon), 0.18°C per decade

(winter), and 0.23°C per decade (post-monsoon) (Zaman et al. 2013; IPCC 2023; Figure 1). The average annual rainfall increased by 4.20 mm/year, while pre-monsoon rainfall increased by 1.35 mm/year (Alam et al. 2023). Another finding from 1981 to 2010 was that the annual maximum temperature increased compared to 1971-2000, particularly in Bangladesh's southeastern and northeastern regions, followed by the center and southern areas (Figure 2) (Khatun et al. 2016). These forecasts warn Bangladesh of a surge in hot days, heat waves, extended dry periods, and increased drought threats. Thus, climate change is believed to be deteriorating, threatening crop output, the survival of plant and animal kingdoms, forestry, biodiversity, and global sustainability, among other issues.
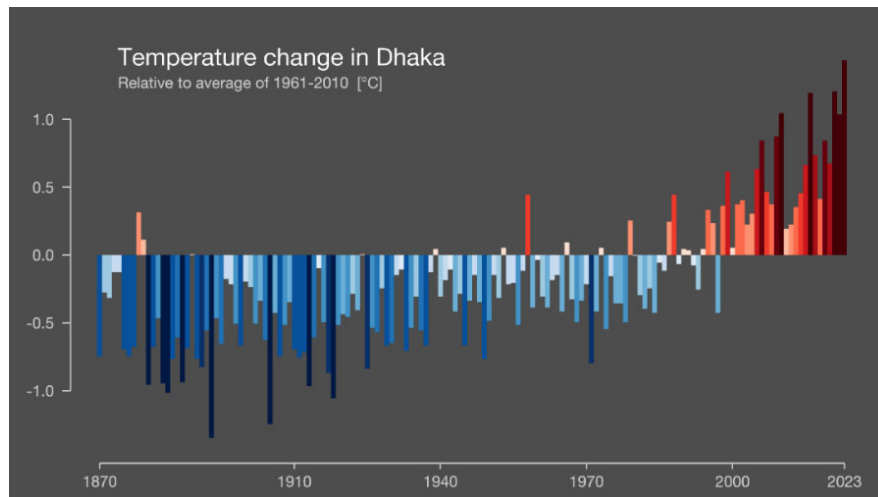

Figure 1. The temperature development of Bangladesh from 1870 to today. Data from Berkeley Earth & ERA5-Land, University of Reading, UK, Graphics Link: https://showyourstripes.info/c/asia/bangladesh/dhaka, Retrieved 20 July 2024.

In Bangladesh, wheat sowing is from 15 to 30 Nov. However, if sown in Dec, the reproductive and grain-filling stages coincide with mid-February to mid-Mar, a period of high temperatures. This terminal heat stress, with temperatures above 30°C during the reproductive development stage, severely impacts productivity (Figure 2) (Ding and Yang 2022; Mao et al. 2023). High temperatures during the grain filling stage, combined with rising mean temperature due to global warming, significantly affect grain production, leading to a reduction in the period of grain growth, improper or shrinkage of grains, and an overall decrease in wheat yield (Figure 2; Alam et al. 2013a; Hossain et al. 2019; 2023). Heat stress is a major cause of yield decrease, affecting around 36 million hectares of land in temperate regions globally (Elahi et al. 2022). Heat stress is a significant threat to wheat farming in South Asia. Most crops with increased yields are investigated using traditional breeding techniques and cultivated in ideal conditions at the right time of sowing. Because of this, most wheat cultivars made available for use in agriculture do not have heat stress tolerance (Hossain et al. 2023). Given the situation's urgency, biotechnological studies that genetically alter kinds with vast adaptability must proceed quickly to generate heat-tolerant genotypes that can withstand varying temperatures.
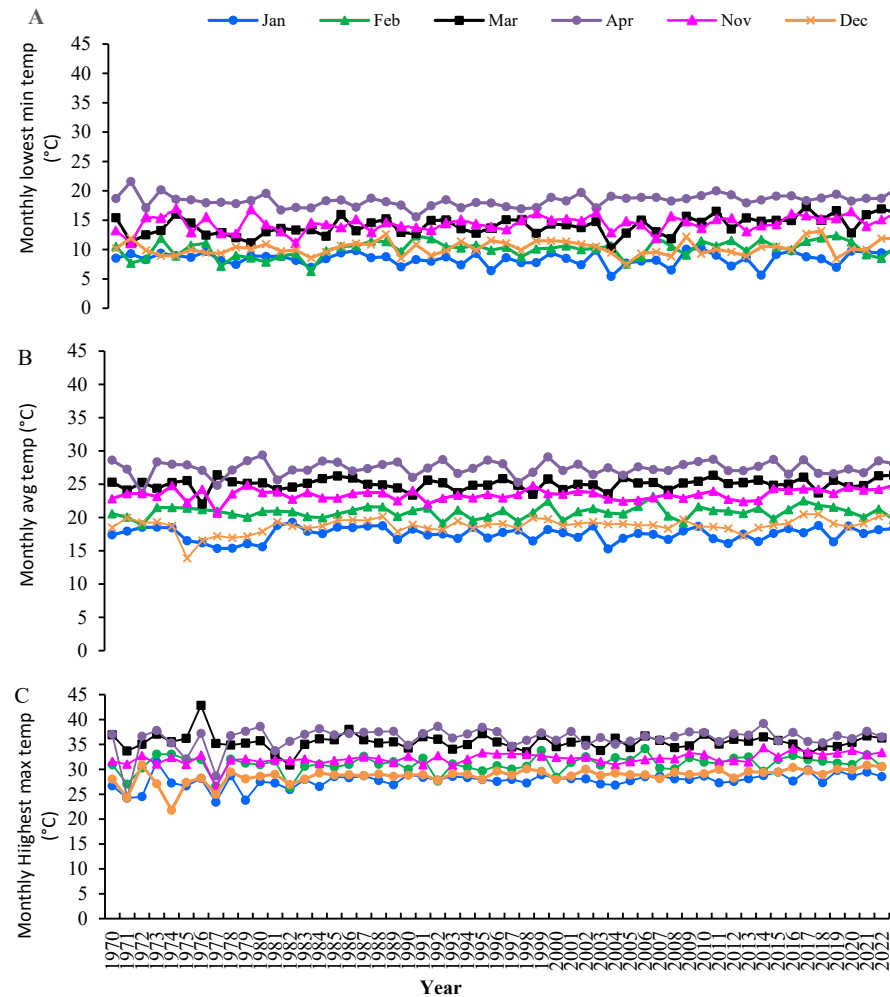
Figure 2. The monthly minimum (A), average (B), and maximum temperature (°C) of Bangladesh for January, February, March, April, November, and December in the years 1970 to 2022. Monthly data are the average of seven divisions (Dhaka, Rajshahi, Barishal, Sylhet, Rangpur, and Mymensingh Division) and one region (Dinajpur) of Bangladesh (Average = avg, Minimum = Min, maximum = Max, Temperature = Temp) (BMD 2024).

The first step in the breeding of heat-tolerant varieties involves research studies that address a range of topics, including mitigating the effects of heat, characterizing wheat varieties molecularly and biochemically, and classifying the varieties according to their resistance to heat stress (Chaudhary et al. 2020). During the 2022-2023 growing season, 1.16 million metric tons of wheat grains were produced in Bangladesh in around 0.311 million hectares of land (DAE 2023; BBS 2023). Some parts of Bangladesh are vulnerable to high temperatures, which could be impacted by climate change, despite its significance to global output. Wheat has a vast genome (~17 Gb) for bread wheat) (Walkowiak et al. 2020). Hence, heat tolerance is a complicated and quantitative feature controlled by several genes. Numerous studies on molecular processes of heat tolerance and molecular breeding for heat stress have been conducted to address these issues. Several molecular markers and quantitative trait loci (QTLs) have recently been linked to the genes that control heat signaling processes (Chaudhary et al., 2020). Significant progress has been made in identifying interest genes (Yıldırım et al. 2013; Govindaraj et al. 2018). These advancements enabled the production of future wheat crops that are heat-stress resistant. A variety of molecular markers have evolved to help achieve this goal. The most notable are DNA markers that use polymerase chain reaction (PCR). Wheat genetic characterization studies utilized PCR-based markers, such as amplified fragment

length polymorphisms, sequence-tagged microsatellite site markers (SSRs) (Ateş Sönmezoğlu and Terzi 2018), and chloroplast-specific microsatellite markers (Tomar et al., 2013). Golabadi et al. (2011) employed microsatellite markers like TGW and the harvest index to discover QTLs associated with yield trait competence. In a separate study, Ramya et al. (2015) described the physiological and genetic characteristics of 24 current wheat genotypes for use in breeding studies to investigate drought and high-temperature tolerance. Previous research has shown that SSR markers can be utilized to determine heat tolerance in wheat accurately.

After several agronomic parameters were evaluated in the field, the HSI could be used to identify genotypes of wheat that yielded well under heat stress. Wheat genotypes with improved relative performance in yield components, grain yield, and HSI were quantified through high-temperature tolerance characterization (Hossain et al. 2021; Shenoda et al. 2021). The application of HSI and performance in ILS-heat-stressed conditions have also been documented in several earlier research (Bhusal et al. 2017). Genotypes with contrasting features must be found to perform genetic analyses, such as QTL investigations. This study aimed to characterize DNA as fingerprinting for future intellectual property and identify heat-responsive wheat genotypes.

Materials and Methods

Plant Materials

To evaluate the molecular diversity for terminal heat stress tolerance against 13 SSR markers associated with the trait of interest, fifteen genotypes of bread wheat (*Triticum aestivum* L.) (including 13 varieties and two advanced lines) were employed (Table 1 and Table 2). The Bangladesh Wheat and Maize Agricultural Research Institute (BWMRI), located in Nashipur, Dinajpur, Bangladesh, provided seeds of every genotype. Table 1 provides a summary of the genotypes' pedigrees and salient features.

Table 1. List of fifteen wheat genotypes with their pedigrees.

Variety	Pedigree	Year of release	Life cycle (d)	Salient features
BARI Gom 25	ZSH 12/HLB 19//2*NL297	2010	102-110	• Heat and salinity tolerance. It can tolerate 8.0-10 dS/m salinity at the seedling stage • High yielding (3.8-5.0 t ha^{-1}) • Resistant to leaf rust (LR) and Bipolaris leaf blight (BpLB) diseases
BARI Gom 26	ICTAL 123/3/RAWAL 87//VEE/HD 2285 BD(JO)9585-0JO-3JE-0JE-0JE-HRDI-RC5DI	2010	104-110	• Heat tolerance • Suitable for late sowing • High yielding (4.0-5.0 t ha^{-1}) • Resistant to LR and tolerant to stem rust (Ug99 race) and moderately resistant to BpLB
BARI Gom 27	WAXWING*2/VIVISTI CGSS01BOOO56T-099Y-099 M-099 M-099Y-099 M-14Y-0B	2012	105-110	• High yielding (3.5-5.4 t ha^{-1}) • Resistant to LR and stem rust (Ug99 race) and moderately resistant to BpLB

BARI Gom 28	CHIL/2*STAR/4/BOW/CROW//BUC/PVN/3/2*VEE#10 CMSS95Y00624S-0100Y-0200M-17Y-010 M-5Y-0 M	2012	102-108	• Early maturing and heat tolerance. • Suitable for late sowing • High yielding (4.0-5.5 t ha^{-1}) • Resistant to LR and moderately resistant to BpLB
BARI Gom 29	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	• Moderately tolerant to heat stress • High yielding (4.0-5.0 t ha^{-1}) • Resistant to leaf and stem rust (Ug99 race) and moderately resistant to BpLB
BARI Gom 30	BAW 677/Bijoy BD(JA)1365S-0DI-15DI-3DI-HR12R3DI	2014	100-105	• Heat tolerance and suitable for late sowing • High yielding (4.0-5.5 t ha^{-1}) • Resistant to leaf rust and moderately resistant to BpLB
BARI Gom 31	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}
BARI Gom 32	SHATABDI/GOURAB BD(DI)1686S-0DI-1DI-0DI-0DI-3DI	2017	95-105	• Early maturing, heat tolerant, short stature • Resistant to LR and tolerant to spot blotch • Has tolerance to wheat blast (10-12% infection at Jessore) • Yield:4.6-5.0 t ha^{-1}
BARI Gom 33	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)

WMRI Gom 1	BARI Gom 21 (Shatabdi)/BARI Gom 24	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
WMRI Gom 2	BARI Gom 26/BARI Gom 25	2021	108-115	- Grain in amber color - Panicle tall in length, 45-48 grains/panicle, 1000-grain weight 45-50 g - Resistant to LR and tolerant to heat stress - Yield: 4.5-5.8 t ha^{-1}
WMRI Gom 3	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 Gom 21/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	AATGGTATCTAT TCCGACCCG	CATCCCTAGGCC ACTCTGC	5A	60
2	Gwm325	HSI grain filling duration HSI kernel weight	TTTTTACGCGTCA ACGACG	TTTCTTCTGTCGT TCTCTTCCC	6D	60
3	Xgwm294	HIS single kernel weight of the main spike	GCAGAGTGATCA ATGCCAGA	GGATTGGAGTTA AGAGAGAACCG	2A	55
4	Gwm268	HSI kernel weight	TTATGTGATTGCG TACGTACCC	AGGGGATATGTT GTCACTCCA	1B	55

5	Xwmc407	Grain-filling duration	CATATTTCCAAA TCCCCAACTC	GGTAATTCTAGG CTGACATATGCT C	2A	61
6	Xcfa2129	HIS single kernel weight of the main spike	ATCGCTCACTCA CTATCGGG	GTTGCACGACCT ACAAAGCA	1A, 1B, 1D	60
7	gwm11	Grain-filling duration	GTGAATTGTGTCT TGTATGCTTCC	GGATAGTCAGAC AATTCTTGTG	1A, 1B	50
8	Xcfd43	Grain-filling duration	CCAAAAACATGG TTAAAGGGG	AACAAAAGTCG GTGCAGTCC	2D	60
9	Xgwm356	HSI single kernel weight of the main spike	CCAATCAGCCTG CAACAAC	AGCGTTCTTGGG AATTAGAGA	2A, 6A, 7A	55
10	Xbarc137	Waxiness	CCAGCCCCTCTA CACATTTT	GGCCCATTTCCC ACTTTCCA	1B	52
11	Gwm484	Waxiness	AGTTCCGGTCAT GGCTAGG	ACATCGCTCTTC ACAAACCC	2D	55
12	Gwm293	Grain-filling duration	TCGCCATCACTC GTTCAAG	TACTGGTTCACA TTGGTGCG	5A	55
13	WMC527	HIS kernel weight of the main spike	GCTACAGAAAAC CGGAGCCTAT	ACCCAAGATTGG TGGCAGAA	3A, 3B	61

Experimental Site and Sowing Date

In the Rabi season (Nov to Mar), the genotypes were evaluated at the research field of the Regional Station (RS), BWMRI, Joydebpur, Gazipur, which is located in the agro-ecological zone 28 (Madhupur Tract). This region is distinguished by intricate relief and soils that have grown on the Madhupur Clay. The terrain consists of undulating hills and splintered terraces with deep, narrow valleys. The heavy clays in the valleys are dark grey, highly acidic, and have poor fertility levels, organic matter, and moisture-holding capacity. Seeds were sown on two dates viz. Nov 21 (irrigated timely sowing or ITS) and Dec 21 (irrigated late sowing or ILS). The laboratory experiments were conducted at the Biotechnology Division, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur, and the Molecular Laboratory, RS, BWMRI, Gazipur.

Extraction of DNA and SSR Analysis

Using a modified CTAB approach, genomic DNA was isolated from fresh leaves of fifteen wheat genotypes. 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 ground 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 4°C 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 that Roder et al. (1998) adhered to.

Heat Susceptibility Index (HSI)

The impact of heat stress on TGW and grain yield was assessed using the heat susceptibility index (HSI). The following formula was used to calculate the HSI in the manner outlined by Paliwal et al. (2012):

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

where

X represents TGW and grain yield

$X_{\text{heat stress}}$ 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).

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

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

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

Statistical Analysis

Only one locus was assigned to each band. The Unwaited Pair Group Method with Arthemetic Mean (UPGMA), outlined for the shared allele, was used to quantify the genetic distances between the genotypes after all the scorable loci were considered to create a bivariate 1-0 data matrix. Values were designed to estimate genetic diversity using PowerMarker software, dendrograms, and polymorphism information content (PIC).

Results

The 13 SSR primers used in the study are listed in Table 2. Table 3 shows the primers' allele counts and sizes. The primers detected 2.0 to 8.0 alleles per genotype. Gwm293, with eight alleles, was the most polymorphic microsatellite marker, followed by Xgwm356, which had seven alleles (Table 3; Figure 3). Screening 15 genotypes with 13 SSR markers yielded 51 polymorphic alleles, averaging 3.92 per locus. Xcfd43 had the fewest alleles, only two alleles. The PIC values of the studied microsatellite markers ranged from 0.33 (Xwmc407) to 0.83 (Xgwm356), with an average of 0.57 (Table 3; Figure 3). The Xgwm294, Xcfa2129, and Gwm293 primers had the highest heterozygosity (He) values of 1.00, while the lowest values ranged from 0.33 (Xwmc407) to 0.83 (Xgwm356), with an average PIC value of 0.57. The primers gwm291, gwm325, Xwmc407, gwm11, Xcfd43, and Xbarc137 all had He values of 0.0. heterozygosity was the most commonly employed concept for evaluating population genetic variation (Table 3). For the microsatellites included in this investigation, PIC exhibited a strong positive connection with the number of alleles (Table 4). Table 4 displays 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.

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	2.00-8.00	-	2.00 - 105	0.200 - 0.714	0.49-0.85	0.00 - 1.00	0.33-0.83

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

	BARI Gom 25	BARI Gom 26	BARI Gom 27	BARI Gom 28	BARI Gom 29	BARI Gom 30	BARI Gom 31	BARI Gom 32	BARI Gom 33	BAW 1147	BAW 1290	Na di 2	WMRI Gom 1	WMRI Gom 2	WMRI Gom 3
BARI Gom 25	0.000														
BARI Gom 26	0.150	0.000													
BARI Gom 27	0.545	0.500	0.000												
BARI Gom 28	0.583	0.500	0.458	0.000											
BARI Gom 29	0.583	0.600	0.591	0.250	0.000										
BARI Gom 30	0.667	0.700	0.591	0.333	0.083	0.000									
BARI Gom 31	0.708	0.650	0.727	0.542	0.292	0.292	0.000								
BARI Gom 32	0.714	0.786	0.500	0.714	0.571	0.571	0.571	0.000							
BARI Gom 33	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				

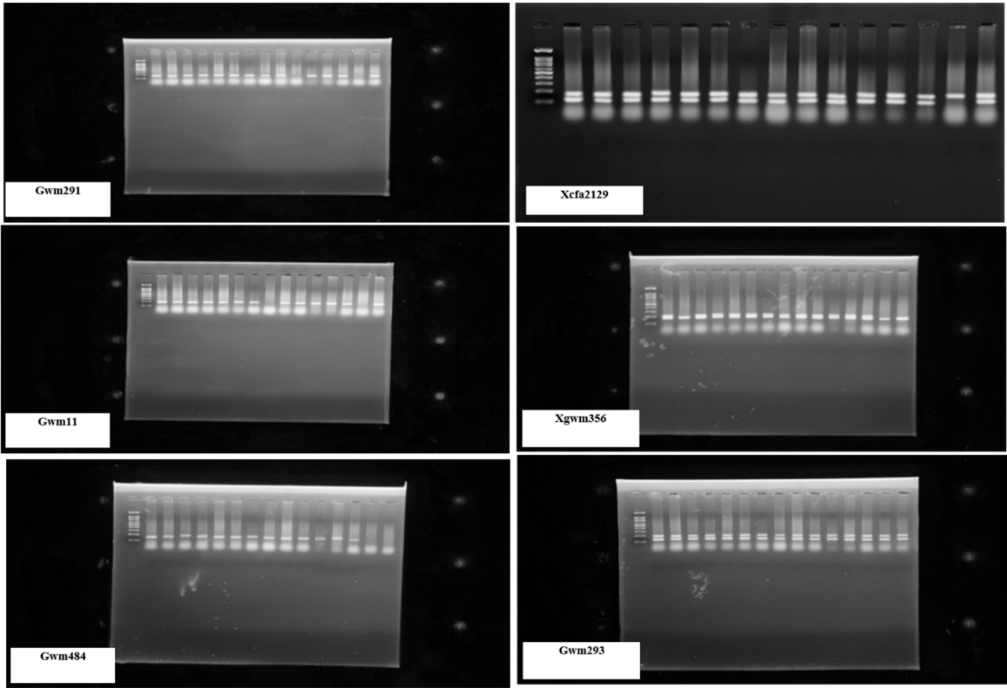|														
Nadi 2	0.800	0.800	0.800	0.900	0.700	0.700	0.700	0.400	0.500	0.000	0.200	0.000		
WMRI Gom 1	0.714	0.786	0.500	0.786	0.500	0.500	0.286	0.333	0.167	0.875	0.700	0.625	0.000	
WMRI Gom 2	0.700	0.813	0.778	1.000	0.800	0.800	0.600	0.417	0.333	0.875	0.500	0.500	0.167	0.000
WMRI Gom 3	0.800	0.889	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

Figure 3. SSR marker profiles of bread wheat genotypes using the SSR primers gwm291, Xcfa2129, gwm11, Xgwm356, Gwm484, and Gwm2.

This outcome was in line with earlier research by Herrera et al. (2008), who also found a significant genetic diversity at each SSR locus based on the number of alleles found using microsatellite markers (Table 4). A dendrogram was drawn out based on the genetic distance between common alleles derived from 15 genotypes and 51 alleles (Figure 4). 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 (Figure 4). 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. TGW and grain yield under ILS and ITS conditions, the mean percentage change in TGW and grain yield under ILS compared to ITS, and the values of the same parameters for each cluster member. The HSI, TGW, and grain yield under stress conditions, as well as the percent decrease in TGW and grain yield under the ILS over the ITS, were the three parameters that divided the two major groups obtained from the cluster analysis as a measure of heat tolerance under field conditions (Tables 5; Table 6). The HSI was assessed for TGW and grain yield to determine which genotypes were heat-tolerant and heat-susceptible. The grain yield varied from 0.74 to 1.25, whereas the HSI for TGW varied from 0.772 to 1.23. Heat-tolerant genotypes were identified using these data. Heat-stress tolerance was correlated with a low HSI (HSI<1) (Table 5;

Fischer and Maurer, 1978). Different genotypes were characterized as having high heat tolerance (HSI<0.50), moderate heat tolerance (HSI = 0.50-1.00), or heat sensitivity (HSI>1.00) based on the value and direction of desire (Khanna-Chopra and Viswanathan 1999 and Singh, et al. 2011). Table 5 displays the mean HSI values for TGW under ITS and ILS conditions, the mean percentage change in TGW and grain yield under the ILS compared to ITS, and the values of the same parameters for each cluster member. The HSI, TGW, and grain yield under stress conditions, as well as the percent decrease in TGW and grain yield under the ILS over the ITS, were the three parameters that separated the two major groups obtained from the cluster analysis as a measure of heat tolerance under field conditions (Table 5).

Table 5. Summary of wheat genotype clusters according to morphophysiological traits (TGW).

Cluster	Genotypes	HSI	TGW ITS	TGW ILS	%TGW decrease	HSI	TGW ITS	TGW ILS	%TGW decrease
Group A									
Cluster I	BARI Gom 25	0.887	49.00	43.87	10.47	0.887	49.00	43.87	10.47
	BARI Gom 26	0.885	48.05	43.03	10.45				
	BARI Gom 27	0.870	42.85	38.45	10.27				
Cluster II	BARI Gom 28	0.772	45.00	40.90	9.11	0.772	45.00	40.90	9.11
	BARI Gom 29	0.892	42.35	37.89	10.53				
	BARI Gom 30	0.823	46.80	42.25	9.72				
	BARI Gom 31	0.909	42.75	38.16	10.74				
Group B									
Cluster III	BWMRI Gom 3	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 Gom 32	1.169	48.55	41.85	13.80	1.169	48.55	41.85	13.80
	BARI Gom 33	1.061	48.85	42.73	12.53				
	BWMRI Gom 1	1.129	48.85	42.34	13.33				
	BWMRI Gom 2	1.043	49.30	43.23	12.31				

Table 6. Summary of wheat genotype clusters according to morphophysiological traits (grain yield).

Cluster	Genotypes	HIS	Yield ITS	Yield ILS	% Yield decrease	HSI	Yield ITS	Yield ILS	% Yield decrease
Group A									
Cluster I	BARI Gom 25	0.903	2.68	2.31	13.81	0.903	2.68	2.31	13.81
	BARI Gom 26	0.964	2.17	1.85	14.75				
	BARI Gom 27	0.960	2.52	2.15	14.68				
Cluster II	BARI Gom 28	0.742	2.38	2.11	11.34	0.742	2.38	2.11	11.34
	BARI Gom 29	0.833	2.59	2.26	12.74				
	BARI Gom 30	0.756	2.68	2.37	11.57				
	BARI Gom 31	0.930	2.67	2.29	14.23				
Group B									

Cluster III	BWMRI Gom 3	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 Gom 32	1.050	2.49	2.09	16.06	1.050	2.49	2.09	16.06
	BARI Gom 33	1.004	2.54	2.15	15.35				
	BWMRI Gom 1	1.102	2.61	2.17	16.86				
	BWMRI Gom 2	1.056	2.60	2.18	16.15				

Figure 4. Shows a Dendrogram generated through UPGMA analysis showing the genetic relationships among fifteen wheat genotypes. The names of the genotypes are given at the ends of the branches.

Three genotypes, BARI Gom 25, BARI Gom 26, and BARI Gom 27, 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). The genotypes that made up Cluster II were BARI Gom 28, BARI Gom 29, BARI Gom 30, and BARI Gom 31.

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 Gom 3, 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). Four genotypes comprised Cluster IV: BARI Gom 32, BARI Gom 33, BWMRI Gom 1, and BWMRI Gom 2. 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. The outcomes of Ali et al. (2013), Pinto et al. (2010), and Sadat et al. (2013) agreed with these results.

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 produced distinct bands for heat-tolerant genotypes, indicating that they may be used to increase heat tolerance. Wheat varieties with higher yields under abiotic stress can be developed with the help of phenotypic and molecular data integration (Haliloglu et al. 2022).

The 13 SSR markers resulted in 51 polymorphic bands with an average PIC of 0.57. The number of bands per marker ranged from 2 (Xcfd43 and Xwmc407) to 7 (Xgwm356), with an average of 3.92 bands. PIC values ranged from 0.38 for Xcfd43 to 0.83 for Xgwm356, showing that some SSRs are informative. 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 shows that distinct chromosomes contain SSR markers linked to heat tolerance. With an average of 0.37 in wheat genotypes examined, low heterozygosity showed limited genetic variation, according to the SSR markers employed in this investigation. 13 loci with PIC values larger than 0.50 were deemed informative among the PIC values for the primers utilized. The PIC can be used to assess a crop's genetic variation. According to Nagy et al. (2012) and Ramadugu et al. (2015), a locus with a PIC greater than 0.5 has high diversity, while a PIC less than 0.25 indicates low diversity. The mean PIC for the SSR markers employed in this investigation was 0.57, ranging from 0.33 to 0.83 (Table 3). As a result, most of the primers utilized in this investigation provided valuable information. The results showed that the SSRs were suitable markers for terminal heat stress tolerance selection using molecular plant breeding. Mourad et al. (2020) proposed that the number of alleles at each locus and their calculated PIC values be examined simultaneously as part of an objective assessment of genetic diversity in genotyping collections.

All four clusters had distinct traits. Cluster IV exhibited the most excellent mean HSI value (>1.00) and the most significant drop in TGW under ILS (sowing on 21 December) against ITS (sowing on 21 November) (Table 5). In contrast, cluster III exhibited the highest mean HSI value and the most significant drop in grain output under ILS compared to ITS (Table 6). However, BARI Gom 25 and BARI Gom 30 showed more considerable genetic potential for yield under ILS, beating the other genotypes in group A. BAW 1147 was classified as heat-sensitive due to its greater HSI for grain yield (Table 6). Alam et al. (2013c; 2013b; 2014) reported similar outcomes. According to Table 6, group A's genotypes BARI Gom 25, BARI Gom 26, BARI Gom 27, BARI Gom 28, BARI Gom 29, BARI Gom 30, and BARI Gom 31 were suitable for ILS circumstances. 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 (2.19 kg) in the ILS despite being in the heat-sensitive group. In contrast, BARI Gom 26 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 (1.85 kg) within the heat-tolerant group. This was comparable to the grain yield (1.63 kg) of WMRI Gom 3 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 has a complex impact on yield, and the genotype-environment interaction plays a crucial role in yield expression. This examination, conducted in a field with apparent weather fluctuations, is consistent with the findings of Haliloglu et al. (2022). Because temperatures above the optimum (21.3±1.27°C) during the grain-filling stage significantly impact wheat output, agricultural research prioritizes developing wheat types that can withstand heat. Genotypes that can withstand terminal heat stress or mature early without suffering appreciable yield losses must be developed or identified (Alam et al. 2014; Shenoda et al. 2021). The molecular and genetic methods used in this work demonstrated that marker-assisted breeding, cloning, and the characterization of genetic determinants for increased heat tolerance are all supported by DNA polymorphisms that confer thermotolerance. Because there were few abnormalities, the SSR markers helped classify wheat genotypes as either terminal heat stress tolerant or susceptible. Through this study, heat-tolerant and heat-sensitive wheat genotypes were identified, and the DNA fingerprinting of the genotypes was characterized to be established as future evidence of their intellectual property.

Conclusions

Genetic similarity data is crucial for maintaining genetic diversity and improving breeding operations. Cultivars with varied DNA profiles likely contain distinct genes, enhancing genetic variation and heat tolerance assessments. 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 most significant genetic distances were between BAW 1290 and BARI Gom 28 (0.929), followed by Nadi 2 and BARI Gom 28 (0.900), WMRI Gom 3 and BARI Gom 26 (0.889), and the lowest distance (0.000) was between Nadi 2 and BAW 1147. Cluster I genotype showed moderate heat tolerance with HSI values for the 1000-grain weight (TGW) and grain yield ranging from 0.885 to 0.964, closely matching Cluster II, which included BARI Gom 28, BARI Gom, BARI Gom 29, BARI Gom 30 and BARI Gom 31. Cluster III in Group B had higher HSIs and significant TGW and grain yield reductions under heat stress (1.038 - 1.218). Cluster IV genotypes also showed heat sensitivity (HSI > 1.00). Group A consisting of seven genotypes (Cluster I & II) (BARI Gom 25, BARI Gom 26, BARI Gom 27, BARI Gom 28, BARI Gom 29, BARI Gom 30, and BARI Gom 31 were identified as suitable for cultivation under heat-stressed conditions. This study analyzed DNA fingerprinting and identified heat-tolerant and heat-sensitive wheat genotypes, providing valuable insights for future molecular breeding programs focused on heat tolerance.

Conflicts of Interest: All authors declare that they have no conflict about the research paper.

References

Alam E, Hridoy A-EE, Tusher SMSH et al. (2023) Climate change in Bangladesh: Temperature and rainfall climatology of Bangladesh for 1949-2013 and its implication on rice yield. **PLoS ONE** 18: e0292668.

Alam MN*, Islam MZ, Mamun MAA, Md. MSN, Hossain MM, et al. (2023). Searching high yielding durum wheat genotype (s) through the assessment of the physiology, yield, and yield contributing attributes. **International Journal of Agronomy and Agricultural Research** 23(3): 1-9.

Alam MN, Akhter MM, Hossain MM, Mahbubul SM (2013b). Phenological changes of different wheat genotypes (*Triticum aestivum* L.) in high temperature imposed by late seeding. **Journal of Biodiversity and Environmental Sciences** 3(8): 83-93.

Alam MN, Akhter MM, Hossain MM, Rokonuzzaman (2013c) Performance of different genotypes of wheat (*Triticum aestivum* L.) in heat stress conditions. **International Journal of Biosciences** 3(8): 295-306. http://dx.doi.org/10.12692/ijb/3.8.295-306

Alam MN, Bodruzzaman M, Hossain MM, Sadekuzzaman M (2014) Growth performance of spring wheat under heat stress conditions. **International Journal of Agronomy and Agricultural Research** 4(6): 91-103.

Alam MN, Mannaf MA, Sarker MAZ, Akhter MM (2013a) Effect of terminal high temperature imposed by late sowing on phenological traits of wheat (*Triticum aestivum* L.). **International Journal of Agronomy and Agricultural Research** 3(3): 6-10.

Alam MN, Yang L, Yi X, Wang Q-F*, Robin AHK* (2021) Role of melatonin in inducing the physiological and biochemical processes associated with heat stress tolerance in tall fescue (*Festuca arundinaceous*). **Journal of Plant Growth Regulation** 41: 2759–2768 (2022). https://doi.org/10.1007/s00344-021-10472-6

Alam MN, Zhang L, Yang L, et al. (2018a) Transcriptomic profiling of tall fescue in response to heat stress and improved thermotolerance by melatonin and 24-epibrassinolide. **BMC Genomics** 19: 224. doi: 10.1186/s12864-018-4588-y

Ali RA, Kelestanie A, Asadi A, Mirfakhraei SR, and Abasi AR (2013) Genetic Diversity in Twenty Bread Wheat Cultivars Using Microsatellite Markers. **International Journal of Agronomy and Plant Production** 4(8): 1920–1927.

Ates So¨nmezog˘lu O¨ and Balkan AS (2014) Molecular and biochemical analysis of durum wheat genotypes to examine carotenoid pigment content and lipoxygenase enzyme activity. **Cereal Research Communications** 42(2): 218-228.

Ateş Sönmezoğlu Ö and Terzi B (2018) Characterization of some bread wheat genotypes using molecular markers for drought tolerance. **Physiological and Molecular Biology of Plants** 24:159-166.

Bala S, Asthir B, Bains NS (2014) Effect of terminal heat stress on yield and yield attributes of wheat. **Indian Journal of Applied Research** 4(6): 1-2.

Barrett BA and Kidwell KK (1998) AFLP-based genetic diversity assessment among wheat cultivars from The Pacific Northwest. **Crop Science** 38: 1261-1271.

BBS (Bangladesh Bureau of Statistics) (2023) Yearbook of Agricultural Statistics, Bangladesh Bureau of Statistics, Statistics and Informatics Division, Ministry of Planning, Government of the People's Republic of Bangladesh, Agargaon, Dhaka-1207, Bangladesh.

Bhusal N, Sarial AK, Sharma P, Sareen S (2017) Mapping QTLs for grain yield components in wheat under heat stress. **PLoS One** 12:e0189594.

Bita C and Gerats T (2013) Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. **Frontiers in Plant Science** 4: 273.

BMD (Bangladesh Meteorological Department) (2024) Bangladesh Meteorological Department, Ministry of Defense of the Government of Bangladesh, Agargaon, Dhaka, 1207. Weather data link: https://dataportal.bmd.gov.bd/

Botstein D, White R, Skolnick M, Davis R (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American Journal of Human Genetics 32: 314-331

Chaudhary S, Devi P, Bhardwaj A et al. (2020) Identifying and characterizing contrasting genotypes/cultivars for developing crop heat tolerance: current status and prospects. **Frontiers in Plant Science 11**: 587264.

CRP (2024) Climate Risk Profile: Bangladesh (2024): The World Bank Group.

DAE (Department of Agricultural Extension) (2023) Annual Report 2022-23, Department of Agricultural Extension, Ministry of Planning, Government of the People's Republic of Bangladesh, Khamarbari, Farmgate, Dhaka.

Ding Y and Yang S (2022) Surviving and thriving: How plants perceive and respond to temperature stress. **Developmental Cell 57**: 947-958.

dos Santos TB, Ribas AF, de Souza SGH, Budzinski IGF, Domingues DS (2022) Physiological Responses to Drought, Salinity, and Heat Stress in Plants: A Review. **Stresses. 2**: 113-135.

Elahi I, Saeed U, Wadood A, Abbas A, Nawaz H, Jabbar S (2022) Effect of climate change on wheat productivity, in: Ansari (ed) M.U.R. Wheat, IntechOpen, Rijeka.

Farhad M, Kumar U, Tomar V, et al. (2023) Heat stress in wheat: a global challenge to feed billions in the current era of the changing climate. **Frontiers in Sustainable Food Systems 7**: 2023.

Fischer RA and Maurer O (1978) Crop temperature modification and yield potential in dwarfing spring wheat. **Crop Science 16**: 855-859.

Golabadi M, Arzani A, Mirmohammadi Maibody SAM, Sayed Tabatabaei BE, Mohammadi SA (2011) Identification of microsatellite markers linked with yield components under drought stress at terminal growth stages in durum wheat. **Euphytica 177**: 207–221.

Govindaraj M, Pattanashetti SK, Patne N et al. (2018) Breeding cultivars for heat stress tolerance in staple food crops, in: Özden Y, Çiftçi Y Ö, (eds.) Subsequent generation Plant Breeding, IntechOpen, Rijeka.

Gupta OP, Singh AK, Singh A et al. (2022) Wheat Biofortification: Utilizing Natural Genetic Diversity, Genome-Wide Association Mapping, Genomic Selection, and Genome Editing Technologies. **Frontiers in Nutrition 9**: 826131. https://doi.org/10.3389/fnut.2022.826131

Haliloglu K, Turkoglu A, Tan M, Poczai P (2022) SSR-Based Molecular Identification and Population Structure Analysis for Forage Pea (*Pisum sativum* var. *arvense* L.) Landraces. **Genetics 13(6)**: 1086. DOI:10.3390/genes13061086

Hao CY, Zhang XY, Wang LF, Dong YS, Shang XW, Jia JZ (2006) Genetic diversity and core collection evaluations in common wheat germplasm from the northwestern spring wheat region in China. **Molecular Breeding 17(1)**: 69–77.

Hays D, Mason E, Do JH, Menz M, Reynolds M (2007) Expression quantitative trait loci mapping heat tolerance during reproductive development in wheat (Triticum aestivum). In Wheat production in stressed environments Springer Netherlands, 373-382.

Herrera TG, Duque DP, Almeida IP et al. (2008) Assessment of genetic diversity in Venezuelan rice cultivars using simple sequence repeats markers. **Electronic Journal of Biotechnology 11(5)**: 215-226.

Hossain MM, Rahman MM, Islam R et al. (2019) Evaluation of some wheat genotypes growing under heat stress conditions in two environments in Bangladesh. **Journal of Multidisciplinary Science 1**: 1-7.

Hossain MM, Alam MN*, Islam MZ, Ekram MSB, Islam R, Atiquzzaman M (2021) Spring wheat genotypes exhibit physiological and yield potentiality under late sowing conditions in Bangladesh. **International Journal of Agronomy and Agricultural Research** 18 (2): 1-7.

Hossain MM, Alam MN, Hakim MA et al. (2023) Development of short duration, tolerance to high temperature and *bipolaris* leaf blight, and moderately susceptible to blast disease of wheat genotype with trials in various agroecological zones in Bangladesh. **International Journal of Scientific Research Management 11**: 395-410.

IPCC (2018) Summary for Policymakers. In: Global Warming of 1.5°C. An IPCC Special Report in: Masson-Delmotte VP, Zhai H-O, Pörtner D et al. (eds.). Cambridge University Press, Cambridge, UK and New York, NY, USA, 3-24.

IPCC (2021) Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change, in: Masson-Delmotte VP, Zhai A, Pirani SL et al. (eds.). Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 2391.

IPCC (2023) Summary for Policymakers. in: Climate Change 2023: Synthesis Report. Contribution of Working Groups I, II, and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change, in: Core Writing Team LH, Romero J (eds.). Geneva, Switzerland, 1-34.

Joshi AK, Chand R, Arun B et al. (2007) Breeding crops for reduced-tillage management in the intensive, rice-wheat systems of South Asia. **Euphytica 153**: 135–151.

Khan AA, Shamsuddin AKM, Barma NCD, e al. (2014) Screening for Heat Tolerance in Spring Wheat (*Triticum aestivum* L.). **Tropical Agricultural Research and Extension 17(1):** 26-37.

Khanna-Chopra R and Viswanathan C (1999) Evaluation of heat stress tolerance in an irrigated environment of *T. aestivum* and related species. I. Stability in yield and yield components. **Euphytica 106**: 169-180.

Khatun MA, Rashid MB, Hygen OH (2016) "Climate of Bangladesh", MET report, no. 08/2016, ISSN 2387-4201, Climate, Norwegian Meteorological Institute.

Kirigwi FM, van Ginkel M, Brown-Guedira et al. (2007) Markers associated with a QTL for grain yield in wheat under drought. **Molecular Breeding** 20: 401-413.

Kirigwi FM, van Ginkel M, Brown-Guedira et al. (2007) Markers associated with a QTL for grain yield in wheat under drought. **Molecular Breeding** 20: 401-413.

Kumer L, Chhogyel N, Gopalakrishnan T, et al. (2022) Climate change and future of agri-food production, in: Bhat, R. (ed.), Future Foods, Academic Press, 49-79p.

Lu, Liu H, Wu Y, Yan G (2022) Wheat genotypes tolerant to heat at the seedling stage tend to be also tolerant at the adult stage: The possibility of early selection for heat tolerance breeding. **The Crop Journal 10**: 1006-1013. DOI: 10.1016/j.cj.2022.01.005.

Mao H, Jiang C, Tang C et al. (2023) Wheat adaptation to environmental stresses under climate change: Molecular basis and genetic improvement. **Molecular Plant. 16**: 1564-1589.

Mason ER, Mondal S, Beecher WF, Hays DB (2011) Genetic loci linking improved heat tolerance in wheat (*Triticum aestivum* L.) to lower leaf and spike temperature under controlled conditions. **Euphytica** 180:181-194.

Mason R, Mondal S, Beecher FW et al. (2010) QTL associated with heat susceptibility index in wheat (*Triticum aestivum* L.) under short-term reproductive stage heat stress. **Euphytica, 174**: 423-436.

Mickelbart MV and Bailey-Serres HJ (2015) Genetic mechanisms of abiotic stress tolerance that translate to crop yield stability. **Nature Review Genetics 16(4)**: 237–251.

Mohammadi V, Zali AA, Bihamta MR (2008) Mapping QTL for heat tolerance in wheat. **Journal of Agricultural Science and Technology 10**: 261-267.

Mourad AMI, Belamkar V, Baenziger PS (2020) Molecular genetic analysis of spring wheat core collection using genetic diversity, population structure, and linkage disequilibrium. **BMC Genomics 21:** 434.

Nagy S, Poczai P, Cerna´KI, et al. (2012) PICcalc: an online program to calculate polymorphic information content for molecular genetic studies. **Biochemistry and Genetics 50(9-10):** 670–672.

Paliwal R, Roder MS, Kumar U et al. (2012) QTL mapping of terminal heat tolerance in hexaploid wheat (*Triticum aestivum* L.). **Theoretical Applied Genetics 125**: 561-575.

Pandey GC, Rane J, Sareen S et al. (2013) Molecular investigations on grain filling rate under terminal heat stress in bread wheat (*Triticum aestivum L.*). **African Journal of Biotechnology 12(28):** 4439–4445. DOI: 10.5897/AJB12.2751

Pinto S, Chapman SC, McIntyre CL et al. (2010) For canopy temperature response related to yield in both heat and drought environments. **Theoretical Applied Genetics 121(6):** 1001-1021.

Prasad M, Varshney Rk, Roym JK, et al. (2000) The use of microsatellites for detecting DNA polymorphism, genotype identification and genetic diversity in wheat. **Theoretical Applied Genetics 100**: 584-592.

Rahman MA, Chikushi J Yoshida, S Karim, AJMS (2009). Growth and yield components of wheat genotypes exposed to high-temperature stress under control environment. **Bangladesh Journal of Agricultural Research 34(3)**: 361-372.

Ramadugu C, Keremane ML, Hu X et al. (2015) Genetic analysis of citron (*Citrus medica* L.) using simple sequence repeats and single nucleotide polymorphisms. **Scientia Horticulture 195**: 124-137.

Ramya P, Jain N, Singh PK, Singh GP, Prabhu KV (2015) Population structure, molecular and physiological characterization of elite wheat varieties used as parents in drought and heat stress breeding in India. **Indian Journal of Genetics and Plant Breeding 75(2):** 250-252.

Rane J, Pannu RK, Sohu VS et al. (2007) Performance of yield and stability of advanced wheat genotypes under heat-stressed environments of Indo-Gangetic Plains. **Crop Science 47**: 1561-1573.

Roder MS, Korzun V, Wendehake K et al. (1998) A microsatellite map of wheat. **Genetics 149(4)**: 2007-2023.

Sadat S, Saeid KA, Bihamta MR et al. (2013) Marker-assisted selection for heat tolerance in bread wheat. **World Applied Science Journal 21(8)**: 1181–1189.

Sánchez-Bermúdez M, Del Pozo JC, Pernas M (2022) Effects of combined abiotic stresses related to climate change on root growth in crops. **Frontiers in Plant Science 13**: 918537.

Sharma P, Sareen S, Saini M, Shefali (2017) Assessing genetic variation for heat stress tolerance in Indian bread wheat genotypes using morpho-physiological traits and molecular markers. **Plant Genetics Resources 15(6):** 539-547.

Shenoda JE, Sanad MNME, Rizkalla AA et al. (2021) Effect of long-term heat stress on grain yield, pollen grain viability and germinability in bread wheat (*Triticum aestivum* L.) under field conditions. **Heliyon 7**: e07096.

Tomar RS, Deshmukh RK, Naik KB, Tomar SMS (2013) Development of chloroplast-specific microsatellite markers for molecular characterization of alloplasmic lines and phylogenetic analysis in wheat. **Plant Breeding 133(1)**: 12–18.

Walkowiak S, Gao L, Monat C, et al. (2020) Multiple wheat genomes reveal global variation in modern breeding. **Nature 588**: 277–283.

Yıldırım A, Ates So¨nmezog˘lu O¨, Sayaslan A et al. (2013) Marker-assisted breeding of a durum wheat cultivar for gliadin and LMW-glutenin proteins affecting pasta quality. **Turkish Journal of Agriculture and Forestry 37**: 527–533.

Zaman R, Malaker PK, Murad KFI, Sadat MA (2013) TREND analysis of changing temperature in Bangladesh due to global warming. **Journal of Biodiversity and Environmental Science 3:** 32-38.