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## **Morpho-molecular screening of wheat genotypes for heat tolerance**

**Nihar Ranjan Saha<sup>1</sup>, Md.Tariqul Islam<sup>2</sup>, Md. Monirul Islam<sup>3</sup> and Muhammad Shahidul Haque<sup>1\*</sup>**

<sup>1</sup>Department of Biotechnology, Bangladesh Agricultural University, Mymensingh, Bangladesh.

<sup>2</sup>Plant Physiology Division, Bangladesh Institute of Nuclear Agriculture, Mymensingh, Bangladesh.

<sup>3</sup>Biotechnology Division, Bangladesh Institute of Nuclear Agriculture, Mymensingh, Bangladesh.

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**Wheat (*Triticum aestivum* L.) production in Bangladesh is often impaired by heat stress. Therefore, it has been a priority to develop heat tolerant wheat variety for Bangladesh. An investigation was carried out to evaluate locally cultivated wheat genotypes for heat tolerance based on morpho-physiological and molecular markers. A pot experiment was carried out with ten locally cultivated wheat genotypes. Heat treatment was imposed, 5 days after anthesis, in a plant growth chamber at 35°C and 70% RH for 3 days. The heat stress affected all the yield contributing characters and ultimately led to a reduction in grain yield. BARI gom-29, BARI gom-30 and BARI gom-28 emerged as heat tolerant variety on the basis of susceptibility index and tolerance efficiency. While, Shatabdi, BARI gom-23, BARI gom-26 and BARI gom-24 were heat susceptible. Twenty six wheat genotypes were screened for heat tolerance through seven linked SSR markers that generated 44 alleles among the 26 wheat genotypes with an average of 6.28 alleles per locus. Overall polymorphism information content (PIC) and Nei's gene diversity were 0.68 and 0.72, respectively. Similarity indices based unweighted pair group method with arithmetic mean (UPGMA) analysis separated 26 genotypes into five different clusters. Two morphologically identified tolerant genotypes namely BARI gom-29, BARI gom-30 and one moderate genotype BARI gom-22 were grouped in cluster 2. Therefore, these three varieties can be suitable for cultivation in the north-western part of Bangladesh as heat tolerant cultivars.**

**Key words:** Diversity, heat tolerance efficiency, heat susceptibility index, SSR marker.

### **INTRODUCTION**

Wheat (*Triticum aestivum* L.) has a prominent position among the cereals and supplements nearly one-third of the total world population's diet by providing half of the dietary protein and more than half of the calories (Kasana et al., 2016). During the last four decades of the 20th century, the global wheat production is doubled from 3 to

6 billion and by the year 2020 demand for wheat imposed by growing population is forecasted around 950 million tonnes (Kailash et al., 2017). This target will be achieved only if global wheat production is increased by 2.5% per annum (Singh et al., 2011). This increase in wheat production is much more challenging due to a shortage of

\*Corresponding author. E-mail: haquems@bau.edu.bd.

water and changing climate.

Seasonal fluctuations have a potential impact on the crop development and grain yield. The variation in temperature requirements and temperature extremes varies widely for different cultivars of the same species, among species and it varies widely for most crops. Kalra et al., (2008) emphasized the need of studying the response of crops to weather variations for evaluating the impact of seasonal temperature change and estimating yield dependence of temperature rise of crops. Too early sowing of crop produced weak plants with poor root system as the temperature is above optimum whereas delay in sowing leads to irregular germination which results in poor tillering and finally reduction in yield (Yajam and Madani, 2013). Many authors have reported a reduced crop stand, shorter life cycle, reduced tillering, less biomass production, reduced fertilization and grain development, reduced head size, reduction in number of spikes per plant, number of grains per spike and grain weight as the consequences of heat stress, and all these changes are translated in reduction of grain yield/m<sup>2</sup> under heat stress conditions (Moshatati et al., 2012).

Wheat is very sensitive to high temperature (Slafer and Satorre, 1999) and trends in increasing growing season temperatures have already been reported for the major wheat-producing regions (Alexander et al., 2006; Gaffen and Ross 1998; Hennessy et al., 2008). Wheat experiences heat stress to varying degrees at different phenological stages, but heat stress during the reproductive phase is more pronounced than during the vegetative phase due to the direct effect on grain number and dry weight (Wollenweber et al., 2013).

Yield and yield components in stress condition, are still the most effective tools for stress evaluation (Ozkan et al., 1998). For exploitation of genetic variations to improve stress tolerance and development of stress tolerant cultivars, plant breeders mainly relies on selection of different genotypes under environmental stress conditions (Khan et al., 2014). In spite of several screening methods in many crops and development of selection criteria by different researchers, very few were reported for screening heat tolerant genotypes in wheat. Stress indices based on loss of yield under stress conditions in comparison to normal conditions have been used for screening stress tolerant genotypes. Stress susceptibility index (SSI) was proposed as a ratio of genotypic performance under stress and non-stress conditions and was suggested for measurement of yield stability that apprehended the changes in both potential and actual yields in variable environments (Fischer and Maurer, 1978). Bansal and Sinha (1991) proposed to use SSI and grain yield/m<sup>2</sup> as stability parameters to identify drought resistant genotypes of wheat. Sood et al., (2017) used SSI to distinguish between wheat. With this in mind, it was felt imperative to evaluate some improved wheat genotypes facing high temperatures during and after anthesis under field conditions to identify genotypes that have high yield potential in both relatively favourable and

high-temperature environments for using in a breeding program.

Marker-assisted selection (MAS) approaches have contributed greatly to a better understanding of the genetic bases of plant stress-tolerance in some crops (Liu et al., 2006; Momcilovic and Ristic, 2007) that led to the enhanced tolerance to abiotic stresses. Synthesis of low molecular weight HSP's (heat shock proteins) synthesis in *T. durum* and the response of different heat tolerant *T. aestivum* genotypes to the enzymes like NRA and Peroxidase can reliably indicate thermo-tolerance. Because of the general complexity of abiotic stress tolerance and the difficulty in phenotypic selection, MAS is considered as an effective approach to improve this kind of tolerance. Sadat et al., (2013) revealed the utility of SSR marker linked with various heat tolerant traits like grain filling duration, Heat Susceptibility Index (HSI)/single kernel weight of main spike, HIS/grain filling duration and HSI/kernel weight under heat stress in MAS for screening wheat genotypes to heat stress. However, limited research has been done to identify genetic markers associated with heat tolerance in different plants and no such efforts have been made in Bangladesh. Thus, there is an urgent need to understand genetic factors affecting heat tolerance as well as to identify new diagnostic markers to be deployed in MAS, which will ensure faster yield gains under heat stress environments. In the present investigation, several locally cultivated wheat genotypes were evaluated with the aim to find heat tolerance based on morph-physiological traits and molecular markers.

## MATERIALS AND METHODS

### Plant materials collection for morphological and molecular screening

Twenty five germplasm were originally collected from Regional Wheat Research Centre (RWRC), Rajshahi and one germplasm (BINA gom-1) was collected from Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, (Table 1). Ten genotypes for morphological screening were selected on the basis of their performance in the experiments of a preliminary screening in the previous year (Billah, 2017). The morphological screening experiment was carried out at the net house, Crop Physiology Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, Bangladesh during the period from November 2017 to March 2018. The molecular experiment was carried out at the Molecular Biology Laboratory; Department of Biotechnology, Bangladesh Agricultural University (BAU), Mymensingh.

### Morphological screening of wheat for heat tolerance

#### Pot preparation

A bulk volume of soil was collected, sun dried, ground and sieved. All kinds of weeds, stubbles and residues of crop and weeds were removed. Each of the pots was filled with 10 kg homogeneous soil. Urea, Muriate of Potash (MP), Triple Super Phosphate (TSP) and Bio-fertilizer were applied according to Fertilizer Recommendation

**Table 1.** List of wheat genotypes for morphological and molecular screening.

S/N	Germplasm name	Year of release	Pedigree
1	BARI gom- 25*	2010	ZSH 12/HLB 19//2*NL297
2	BINA gom- 1	2016	-
3	Aghrani	1987	INIA/3/SN64/P416OE//SN64
4	Akbar	1983	RON/TOB or ROBIN-M/(SIB)TOBARI-66
5	Sourav*	1998	NAC/VEE
6	BARI gom- 20*	1998	TURACO/CHIL
7	Shatabdi	2000	MRNG/BUC//BLO/PVN/3/PJB-81
8	BARI gom- 22*	2005	KAN/6/COQ/F61.70//CNDR/3/OLN/4/PHO/5/MRNG/ALDAN//CNO
9	BARI gom- 23*	2005	NL297*2/LR25
10	BARI gom- 24*	2005	G-162/BL-1316//NL-297
11	BARI gom- 26*	2010	ICTAL123/3/RAWAL87//VEE/HD2285
12	BARI gom- 27	2012	Waxwing*2/Vivitsi
13	BARI gom- 28*	2012	CHIL/2*STAR/4/BOW/CROW//BUC/PVN/3/2*VEE#10
14	BARI gom- 29*	2014	-
15	BARI gom- 30*	2014	-
16	BARI gom- 33	2017	-
17	Barkat	1983	BB/GLL//CARP/3/PVN or BLUEBIRD/GALLO//CARPINTERO/3/(SIB)PAVON-76
18	Durum	-	<i>Triticumturgidum</i> L.
19	KalayanSona	1968	PJ/GB55 or PENJAMO-62(SIB)/GABO-55
20	Kanchan	1983	UP301/C306
21	Kheri	-	-
22	Pavon- 76	1979	VCM//CNO/7C/3/KAL/BB [VICAM-71//CIANO-67/SIETE-CERROS-66/3/KALYANSONA/BLUEBIRD]
23	Protiva	1993	KU SELECTION 12
24	Sonalika	1973	II53.388/AN//YT54/N10B/3/LR/4/B494.A.4.18.2.IY/Y53//3*Y50 or II53-388/ANDES//SIB)PITIC-62/3/LERMA-ROJO-64
25	Sonora- 64	1968	YAKTANA-54//NORIN-10/BREVOR/3/2*YAQUI-54
26	Triticale	2009	-

Here, the names with asterisks (\*) were used in morphological study.

Guide (FRG, 2017).

### Experimental design

The experiment was laid out in a completely randomized design (CRD) with three replications. Thus the total number of pots were 60 (10×3×2) for this experiment. Seven seeds were sown in each pot at a depth of one inch. After successful germination, only three plants were left in each pot as the extra plants were removed.

### Heat stress treatment

After 5 days of anthesis, a set of pots were subjected to heat treatment in plant growth chamber (VS-91G09M-1300C). All of 10 varieties were kept in growth chamber for 3 days at 35°C with 70% RH. After the heat stress, pots were returned to the experimental field where the non-treated plants were kept.

### Morphological characters

The plant height, length of flag leaf, width of flag leaf and flag leaf

area were measured and number of leaves per plant was recorded from three plants of each pot before harvesting and mean value was calculated. Leaf chlorophyll content was recorded by using a portable chlorophyll meter (SPAD-502, Minolta, Japan) and photosynthetic rate was measured from the flag leaf of the plant by portable photosynthesis system (Li-6400XT, LI-COR, USA).

The number of effective tillers was recorded at physiological maturity, the spike length, number of spikelet per spike, numbers of filled and unfilled grains per spike, numbers of filled and unfilled grain per spikelet, number of grains per plant, grain weight per plant, 1000-grain weight, shoot weight, total dry matter and days to harvest were recorded after harvesting from three plants of each pot and mean value of three plants was calculated and used to analyse.

### Harvest index (HI %)

The harvest index was calculated from three days oven dried plant sample according to the following rules:

$$HI (\%) = \frac{\text{Grain yield/Plant}}{\text{Biological yield/Plant}} \times 100$$

### Heat tolerance efficiency

Heat tolerance efficiency (HTE) for total grain yield per plant was calculated by the following formula:

$$\text{HTE (\%)} = \frac{\text{Yield under stress condition/Plant}}{\text{Yield under control condition/Plant}} \times 100$$

### Heat susceptibility index

Heat susceptibility Index (HSI) based total grain yield per plant was calculated by the following formula as suggested by Fischer and Maurer (1978).

$$\text{HSI} = \left(1 - \frac{\text{YS}}{\text{YC}}\right) / \left(1 - \frac{\text{XS}}{\text{XC}}\right)$$

Here, YS=Yield under stress condition (g), YC=Yield under control condition (g), XS=Mean yield of all genotypes under stress condition and XC=Mean yield of all genotypes under control condition.

### Molecular screening of wheat for heat tolerance

#### DNA extraction

Genomic DNA was isolated from 21-day old green leaves using CTAB method with minor modifications (IRRI). Purified DNA was checked for quality and quantity using agarose gel electrophoresis as well as Nano Drop spectrophotometer (Thermo Scientific, www.nanodrop.com). Finally, diluted DNA (50 ng/μl) was used to amplify DNA by SSR markers using eppendorf thermo-cycler. The SSR profiles of the amplified products of five representative primers are shown in Figure 2(A-E).

#### SSR marker genotyping

Thirteen SSR markers linked to heat tolerance were used in screening for heat tolerance wheat variety. Primer name, sequences and corresponding annealing temperatures are listed (Table 2). The polymerase chain reaction (PCR) cocktail including DNA had total volume of 10 μl/reaction (IRRI standard protocol) for SSR analysis, composed of 1.0 μl genomic DNA, 5 μl PCR master mix (Go-taq green master mix, Promega corporation, U.S.A), 0.5 μl forward primer, 0.5 μl reverse primer, and 3 μl nuclease free water. Samples were subjected to the following thermal profile for amplification in a thermo cycler: The reaction mix was preheated at 94°C for 3 min followed by 35 cycles of 30 s. denaturation at 94°C, 45 sec annealing at 55-65°C (based on the annealing temperature of the individual primer) and elongation at 72°C for 2 min. After the last cycle, a final step was maintained at 72°C for 7 min to allow complete extension of all amplified fragments followed by holding at 4°C until electrophoresis.

Visualization of amplification products was accomplished on 8% Polyacrylamide gel in 1 X TAE buffer. The Polyacrylamide gel was stained with ethidium bromide solution for 20-25 min. The stained Polyacrylamide gel was illuminated by UV-trans-illuminator and photographed for assessing the DNA profiles. Only five representative gel pictures have been given in this paper to represent allelic variation at DNA level.

### Data analysis

The data obtained in the morphological study were statistically analyzed using analysis of variance (ANOVA) and least significance difference (LSD). The mean was separated by Duncan's Multiple Range Test (Gomez and Gomez, 1984) using MSTAT-C software. Molecular weights of PCR products were estimated using AlphaEaseFC 4 software and the number of alleles per locus, major allele frequency, genetic diversity and polymorphism information content (PIC) values were determined with the help of a genetic analysis software, POWER MARKER version 3.23 (Liu and Muse, 2005). The allele frequency data from POWER MARKER was used to export the data in binary format (allele presence = "1" and allele absence = "0") for analysis with NTSYS-PC version 2.1 (Rolf 1997). The genetic similarity was calculated using 0/1 matrix and SIMQUAL subprogram (Nei and Li, 1979). The resultant similarity matrix helped to construct dendrograms using Sequential Agglomerative Hierarchical Nesting (SAHN) based unweighted pair group method with arithmetic mean (UPGMA) as implemented in NTSYS-PC (version 2.1) (Rolf, 1997) to infer genetic relationships and phylogeny. For estimating the similarity matrix, null alleles were treated as missing data to reduce the biased genetic or similarity measures (Warburton and Crossa, 2002).

## RESULTS AND DISCUSSION

### Morphological screening of wheat for heat tolerance

An artificial temperature controlled facility (Plant growth chamber; VS-91G09M-1300C) was used to simulate the thermal environment in the present study. Similar treatment methods have been reported in other studies because of its better environmental control (Rehman et al., 2009; Feng et al., 2014). It was observed in this study that heat shock resulted in negative impact on all the morphological and physiological characters, except for the number of unfilled grain spike<sup>-1</sup> and spikelet<sup>-1</sup> which increased due to heat stress (Tables 3 to 5). Mohammadi et al., (2004) reported the effects of post anthesis heat stress on head traits of wheat.

The combined analysis of variance showed significant effect for the source of variation for all traits, indicating that the heat stress influenced the expression of the traits. Grain yield decreased from 10.14 g in favourable conditions, to 7.003 g in the stress condition; hence, an average reduction of 30.99% was estimated (Table 5). In the present study, a grain yield reduction from 17.79 to 57.43% was found (Table 6). However, grain yield reduction ranged from 60 to 95% is reported (Albrecht et al., 2007; Yildirim and Bahar, 2010).

The overall mean of yield components also decreased as a function of the heat stress. The number of grains spike<sup>-1</sup> and spikelet<sup>-1</sup>, number of spikelet spike<sup>-1</sup> and 1000-grain weight was highly affected by the heat stress. All genotypes had the largest decrease for the component number of grains spike<sup>-1</sup>. Number of grains spike<sup>-1</sup> seemed to be the most affected trait by the heat stress. The reduction in the number of grains spike<sup>-1</sup> can be attributed to the heat effect on the differentiation of floral organs, male and female sporogenesis, pollination

**Table 2.** List of the selected primers used for heat tolerance screening in wheat genotypes.

Primer name		Sequence	A <sub>T</sub> (°C)	Amplified band (bp)	References
Xbarc84-3B	F	CGCATAACCGTTGGGAAGACATCTG	64	123	Billah (2017)
	R	GGTGCAACTAGAACGTACTTCCAGTC			
gwm132-6B	F	TACCAAATCGAAACACATCAG G	60	116-118	Najeb et al. (2011)
	R	CATATCAAGGTCTCCTTCCCC			
Xgwm285-3B	F	ATGACCCTTCTGCCAAACAC	60	223	Billah (2017)
	R	ATCGACCGGGATCTAGCC			
Xgwm428-3A	F	AGCGTTCTTGGGAATTAG AGA	60	133-137	Najeb et al. (2011)
	R	CCAATCAGCCTGCAACAA C			
Xgwm577-7B	F	ATGGCATAATTTGGTGAATT G	55	136-222	Najeb et al. (2011)
	R	TGTTTCAAGCCCAACTTCTATT			
Xgwm617-6A	F	GATCTT GCGCTGAGAGAGA	60	133	Najeb et al. (2011)
	R	CTCCGATGGACTACTCGCAC			
Xbarc121-7A	F	ACTGATCAGCAATGTCAACTGAA	55	68-221	Najeb et al. (2011)
	R	CCGGTGTCTTTCCTAACGCTATG			

and fertilization (Farooq et al., 2011). High temperatures affect pollen viability, reducing the number of fertilized flowers (Rahman et al., 2009). Similar results were observed by Yildirim and Bahar (2010), the number of grains spike<sup>-1</sup> decreased from 33 in the ideal condition of cultivation to 13 in heat stress condition. At the same heat stress condition, the grain weight reduced from 43 to 14 g. In our study the number of grains spike<sup>-1</sup> decreased from 48.61 in the ideal condition of cultivation to 37.32 in heat stress condition. Under the same conditions, the grain mass reduced from 10.14 to 7.003 g (Table 5).

Reduction in grain weight between 21 and 35% due to heat was reported by Assad and Paulsen (2002). Later, Shah and Paulsen (2003) found that the reduction under stress results from the decrease in the photosynthetic rate of the flag leaf and early leaf senescence. In addition to the damage caused to photosynthesis, starch deposition in grain reduced because the enzymes involved in the biosynthesis of starch are sensitive to high temperatures (Denyer et al., 1994). The yield decrease (19.89 to 57.43%) encountered under heat stress in the present study might be due to the reduction of photosynthetic rate (Tables 3 and 6). One of the main reasons for the deleterious effect of high temperatures is the photosynthesis inhibition (Taiz and Zeiger, 2004). Consequently, carbohydrate reserves dropped and organs lost sugars, causing decrease in production.

#### Effect of heat treatment on heat tolerance parameter of wheat

There was significant interaction between genotypes and

environments for the grain weight. This indicates that the genotypes have different performance when subjected to different environments. For instance, different genotypes express different degrees of heat tolerance. A practical approach to identifying heat tolerant genotypes is to use tolerance indices, which measure the ability of genotypes to maintain their productive potential in stress conditions.

The heat susceptibility index is used in wheat breeding programs for heat tolerance (Khanna-Chopra and Viswanathan, 1999; Rahman et al., 2009; de Oliveira et al., 2011). The reduction in performance when sown under heat-stress conditions from that of the optimum environment was calculated. HSI<1 indicates the tolerance of genotype to heat stress, whereas HSI>1 indicates susceptibility of the genotypes under stress (Fischer and Maurer, 1978). The comparison of these values was used to identify genotypes with least susceptibility to thermal stress. The heat tolerance as measured by heat susceptibility index reflects the stability of performance of genotypes under control and heat stress environments and does not take into account the actual yield obtained under heat stress (Simarjit et al., 2009).

Heat susceptibility index values for the grain weight per plant ranged from 0.57 to 1.86 in the present study. The cultivars BARI gom-28, BARI gom-29, BARI gom-30, BARI gom-25, BARI gom-20 and BARI gom-22 were relatively heat resistant (HSI values <1) and they exhibited smaller yield reductions under heat stress compared with optimum conditions than the mean of all genotypes. On the contrary, the varieties Shatabdi, BARI gom-23, BARI gom-26 and BARI gom-24 were relatively heat susceptible (HSI >1) with concomitant higher yield

**Table 3.** Effect of interaction of heat treatment (35 °C) and genotype on morpho-physiological characteristics.

Genotype	Treatment	PH	NTP	NLP	LFL	WFL	FLA	CC	PR
Shatabdi	Control	79.71 <sup>fg</sup>	15.89 <sup>a</sup>	36.78 <sup>a</sup>	21.61 <sup>g</sup>	1.911 <sup>bc</sup>	39.19 <sup>e-g</sup>	45.48 <sup>bc</sup>	58.47 <sup>cd</sup>
	35°C	72.12 <sup>h</sup>	10.89 <sup>d-f</sup>	21.89 <sup>e-h</sup>	16.70 <sup>h</sup>	1.711 <sup>e-g</sup>	27.17 <sup>m</sup>	32.90 <sup>i</sup>	53.22 <sup>e</sup>
BARI gom-23	Control	79.78 <sup>fg</sup>	13.22 <sup>b</sup>	21.22 <sup>f-h</sup>	18.57 <sup>h</sup>	1.911 <sup>bc</sup>	33.67 <sup>k</sup>	40.13 <sup>f-h</sup>	61.94 <sup>ab</sup>
	35°C	79.56 <sup>fg</sup>	8.223 <sup>k</sup>	15.33 <sup>ij</sup>	17.89 <sup>h</sup>	1.800 <sup>c-f</sup>	30.61 <sup>l</sup>	39.83 <sup>gh</sup>	53.92 <sup>e</sup>
BARI gom-26	Control	86.02 <sup>b-d</sup>	12.45 <sup>bc</sup>	26.00 <sup>de</sup>	29.52 <sup>a</sup>	1.900 <sup>b-d</sup>	52.83 <sup>a</sup>	42.18 <sup>d-f</sup>	63.30 <sup>a</sup>
	35°C	83.00 <sup>e</sup>	8.000 <sup>k</sup>	14.22 <sup>i-k</sup>	26.20 <sup>b-d</sup>	1.934 <sup>bc</sup>	47.67 <sup>b</sup>	39.60 <sup>gh</sup>	53.73 <sup>e</sup>
BARI gom-28	Control	87.61 <sup>ab</sup>	12.11 <sup>bc</sup>	34.56 <sup>ab</sup>	26.70 <sup>bc</sup>	1.722 <sup>e-g</sup>	42.99 <sup>cd</sup>	46.40 <sup>b</sup>	64.67 <sup>a</sup>
	35°C	81.84 <sup>ef</sup>	9.557 <sup>g-j</sup>	17.78 <sup>hi</sup>	23.78 <sup>d-g</sup>	1.655 <sup>f-h</sup>	37.33 <sup>g-i</sup>	39.23 <sup>h</sup>	54.50 <sup>e</sup>
BARI gom-29	Control	86.56 <sup>a-c</sup>	11.78 <sup>cd</sup>	31.00 <sup>bc</sup>	27.49 <sup>ab</sup>	1.856 <sup>b-e</sup>	48.00 <sup>b</sup>	50.99 <sup>a</sup>	63.22 <sup>a</sup>
	35°C	78.34 <sup>g</sup>	9.667 <sup>g-i</sup>	14.89 <sup>ij</sup>	22.57 <sup>fg</sup>	1.700 <sup>e-g</sup>	36.57 <sup>h-j</sup>	41.39 <sup>e-h</sup>	55.55 <sup>de</sup>
BARI gom-30	Control	88.64 <sup>a</sup>	11.67 <sup>c-e</sup>	25.89 <sup>de</sup>	25.82 <sup>b-e</sup>	1.667 <sup>f-h</sup>	40.72 <sup>d-f</sup>	44.17 <sup>cd</sup>	64.53 <sup>a</sup>
	35°C	81.90 <sup>ef</sup>	10.00 <sup>f-h</sup>	14.78 <sup>ij</sup>	23.83 <sup>d-g</sup>	1.522 <sup>h</sup>	34.67 <sup>jk</sup>	42.80 <sup>de</sup>	54.33 <sup>e</sup>
BARI gom-25	Control	88.11 <sup>ab</sup>	9.890 <sup>f-h</sup>	29.89 <sup>cd</sup>	24.34 <sup>c-f</sup>	1.978 <sup>b</sup>	46.73 <sup>b</sup>	43.10 <sup>de</sup>	54.30 <sup>e</sup>
	35°C	86.00 <sup>b-d</sup>	8.113 <sup>k</sup>	14.00 <sup>i-k</sup>	21.43 <sup>g</sup>	1.967 <sup>b</sup>	40.00 <sup>ef</sup>	41.48 <sup>e-g</sup>	41.51 <sup>g</sup>
BARI gom-24	Control	84.33 <sup>c-e</sup>	8.890 <sup>h-k</sup>	25.11 <sup>ef</sup>	26.22 <sup>b-d</sup>	2.222 <sup>a</sup>	55.00 <sup>a</sup>	45.79 <sup>bc</sup>	52.87 <sup>e</sup>
	35°C	78.71 <sup>g</sup>	8.777 <sup>h-k</sup>	15.67 <sup>ij</sup>	23.33 <sup>e-g</sup>	1.989 <sup>b</sup>	44.33 <sup>c</sup>	42.87 <sup>de</sup>	45.89 <sup>f</sup>
BARI gom-20	Control	86.79 <sup>a-c</sup>	10.55 <sup>e-g</sup>	22.89 <sup>e-g</sup>	23.30 <sup>e-g</sup>	1.745 <sup>d-g</sup>	38.87 <sup>f-h</sup>	44.37 <sup>b-d</sup>	59.29 <sup>bc</sup>
	35°C	83.77 <sup>de</sup>	9.000 <sup>h-k</sup>	12.44 <sup>jk</sup>	23.36 <sup>e-g</sup>	1.600 <sup>gh</sup>	35.09 <sup>i-k</sup>	43.07 <sup>de</sup>	55.06 <sup>e</sup>
BARI gom-22	Control	89.00 <sup>a</sup>	8.557 <sup>i-k</sup>	18.67 <sup>g-i</sup>	24.83 <sup>c-f</sup>	1.778 <sup>c-f</sup>	41.62 <sup>de</sup>	41.56 <sup>e-g</sup>	55.95 <sup>de</sup>
	35°C	88.16 <sup>ab</sup>	8.333 <sup>jk</sup>	10.11 <sup>k</sup>	23.13 <sup>fg</sup>	1.800 <sup>c-f</sup>	39.72 <sup>e-g</sup>	33.80 <sup>i</sup>	55.11 <sup>e</sup>
	P-value	**	**	**	*	**	**	**	**
	LSD(0.05)	2.279	1.095	4.186	3.207	0.138	2.387	1.969	3.082
	LSD(0.01)	3.049	1.465	5.602	3.087	0.185	3.195	2.634	4.124
	CV (%)	1.65	6.46	11.99	5.94	4.64	3.56	2.84	3.33

In a column, figures with same letter (s) or without letter do not differ significantly whereas figures with dissimilar letter differ significantly (as per DMRT). \*\*, \* = Significant at 1% and 5%, respectively level of probability, PH= Plant height (cm), NTP= No. of tiller plant<sup>-1</sup>, NLP= No. of leaf plant<sup>-1</sup>, LFL= Length of flag leaf (cm), WFL= Width of flag leaf (cm), FLA= flag leaf area (cm<sup>2</sup>), DB= Days to booting, CC= Chlorophyll content (SPAD reading) and PR= Photosynthetic rate ( $\mu\text{CO}_2\text{cm}^{-2}\text{s}^{-1}$ ).

reduction. Significant differences were observed in HTE and HSI in all the genotypes under stress condition. The HTE in susceptible genotypes ranged from 42.57 to 64.28% while in tolerant genotypes; it ranged from 71.71 to 82.21%. HSI ranged from 1.16 to 1.86 in the susceptible genotypes and 0.57 to 0.91 in tolerant genotypes. Among the susceptible genotypes BARI gom-26 (1.86) had a higher HSI whereas lower HSI was found in BARI gom-24 (1.16). Among the tolerant genotypes, BARI gom-25 had the highest HSI (0.915) and BARI gom-29 had the lowest (0.575).

On the basis of above discussion, under heat stress, the variety BARI gom-28, BARI gom-29, BARI gom-30, BARI gom-25, BARI gom-20 and BARI gom-22 emerged as tolerant to heat based on HSI and HTE. Therefore, these genotypes had low heat susceptibility indicating their specific suitability under late sowing condition. These results are in conformity with those of Khan et al. (2014) concurred that some genotypes have potential to produce high yield even under high temperature. Among these cultivars, BARI gom-29 showed the highest grain yield followed by the cultivars BARI gom-30 and BARI gom-28

**Table 4.** Effect of interaction of heat treatment (35 °C) and genotype on yield contributing characters.

Genotype	Treatment	TDM	NSP	NSLS	LS	NGS	NUS
Shatabdi	Control	39.37 <sup>a</sup>	5.557 <sup>c</sup>	21.44 <sup>bc</sup>	11.72 <sup>b</sup>	48.89 <sup>a-c</sup>	11.56 <sup>c-e</sup>
	35°C	32.24 <sup>cd</sup>	3.667 <sup>f-i</sup>	21.33 <sup>bc</sup>	11.50 <sup>bc</sup>	33.78 <sup>gh</sup>	12.34 <sup>cd</sup>
BARI gom-23	Control	39.34 <sup>a</sup>	4.890 <sup>cd</sup>	20.00 <sup>b-e</sup>	11.14 <sup>b-e</sup>	40.33 <sup>d-f</sup>	12.55 <sup>cd</sup>
	35°C	28.48 <sup>e</sup>	3.890 <sup>e-i</sup>	19.11 <sup>c-g</sup>	11.04 <sup>b-f</sup>	28.33 <sup>h</sup>	13.00 <sup>bc</sup>
BARI gom-26	Control	33.44 <sup>c</sup>	4.667 <sup>c-e</sup>	18.78 <sup>d-g</sup>	10.38 <sup>c-g</sup>	52.33 <sup>a</sup>	8.443 <sup>fg</sup>
	35°C	27.80 <sup>e</sup>	3.443 <sup>hi</sup>	16.67 <sup>g</sup>	9.713 <sup>gh</sup>	32.56 <sup>gh</sup>	10.78 <sup>de</sup>
BARI gom-28	Control	33.66 <sup>c</sup>	6.443 <sup>b</sup>	18.67 <sup>d-g</sup>	9.733 <sup>f-h</sup>	48.44 <sup>a-c</sup>	9.887 <sup>e-g</sup>
	35°C	27.38 <sup>e</sup>	3.000 <sup>ij</sup>	18.00 <sup>e-g</sup>	8.887 <sup>h</sup>	32.45 <sup>gh</sup>	11.11 <sup>c-e</sup>
BARI gom-29	Control	34.81 <sup>bc</sup>	7.333 <sup>a</sup>	19.44 <sup>b-f</sup>	11.40 <sup>b-d</sup>	47.33 <sup>a-c</sup>	11.11 <sup>c-e</sup>
	35°C	27.43 <sup>e</sup>	4.337 <sup>d-h</sup>	18.78 <sup>d-g</sup>	10.20 <sup>c-g</sup>	34.67 <sup>fg</sup>	12.67 <sup>cd</sup>
BARI gom-30	Control	38.93 <sup>a</sup>	7.000 <sup>ab</sup>	18.44 <sup>d-g</sup>	10.69 <sup>b-g</sup>	50.11 <sup>ab</sup>	8.333 <sup>fg</sup>
	35°C	29.06 <sup>e</sup>	4.447 <sup>d-g</sup>	18.11 <sup>e-g</sup>	10.54 <sup>b-g</sup>	37.55 <sup>e-g</sup>	11.67 <sup>c-e</sup>
BARI gom-25	Control	38.17 <sup>a</sup>	4.667 <sup>c-e</sup>	20.89 <sup>b-d</sup>	10.96 <sup>b-g</sup>	42.78 <sup>c-e</sup>	12.56 <sup>cd</sup>
	35°C	27.36 <sup>e</sup>	4.443 <sup>d-g</sup>	19.89 <sup>b-f</sup>	10.83 <sup>b-g</sup>	37.78 <sup>e-g</sup>	15.00 <sup>a</sup>
BARI gom-24	Control	39.96 <sup>a</sup>	4.553 <sup>d-f</sup>	23.67 <sup>a</sup>	13.69 <sup>a</sup>	53.78 <sup>a</sup>	11.78 <sup>c-e</sup>
	35°C	28.94 <sup>e</sup>	2.190 <sup>j</sup>	20.11 <sup>b-e</sup>	11.08 <sup>b-e</sup>	44.33 <sup>b-d</sup>	14.55 <sup>ab</sup>
BARI gom-20	Control	34.36 <sup>c</sup>	4.780 <sup>c-e</sup>	18.11 <sup>e-g</sup>	10.07 <sup>e-h</sup>	48.56 <sup>a-c</sup>	8.113 <sup>g</sup>
	35°C	30.22 <sup>de</sup>	3.557 <sup>g-i</sup>	17.44 <sup>fg</sup>	10.14 <sup>d-g</sup>	44.44 <sup>b-d</sup>	10.11 <sup>ef</sup>
BARI gom-22	Control	37.36 <sup>ab</sup>	4.443 <sup>d-g</sup>	21.45 <sup>bc</sup>	10.46 <sup>b-g</sup>	53.55 <sup>a</sup>	10.22 <sup>ef</sup>
	35°C	30.12 <sup>de</sup>	3.223 <sup>i</sup>	21.67 <sup>ab</sup>	10.08 <sup>d-h</sup>	47.33 <sup>a-c</sup>	12.33 <sup>cd</sup>
	P-value	**	**	*	*	**	*
	LSD(0.05)	2.771	0.837	2.085	1.111	5.679	1.709
	LSD(0.01)	3.708	1.119	2.791	1.486	7.599	2.286
	CV (%)	5.1	11.2	6.45	6.28	8.01	9.08

In a column, figures with same letter (s) or without letter do not differ significantly whereas figures with dissimilar letter differ significantly (as per DMRT). \*\*, \* = Significant at 1% and 5%, respectively level of probability, TDM= Total dry matter (g), NSP= No. of spike plant<sup>-1</sup>, NSLS= No. of spikelet plant<sup>-1</sup>, LS= Length of spike (cm), NGS= No. of grain spike<sup>-1</sup> and NUS= No. of unfilled grain spike<sup>-1</sup>.

with better adaptation to heat. In addition to be more productive, these varieties showed the higher number of tillers per plant, photosynthetic rate, harvest index and low mass reduction for grain yield under heat condition. Billah (2017) found that BARI gom-29 had superior performance under adverse conditions, recommending its cultivation in unfavourable environments. In contrast, Shatabdi, BARI gom-23, BARI gom-26 and BARI gom-24 preformed as susceptible varieties under heat stress condition. Because of these varieties had showed lower yield due to higher yield reduction under stress condition. BARI gom-25, BARI gom-20 and BARI gom- 22 varieties

were intermediate in their performance under heat stressed condition. The study revealed that there are significant differences in performance among genotypes in regard to each trait.

### Molecular screening of wheat for heat tolerance

#### Overall SSR diversity

Data derived from these experiments were analyzed to evaluate the usefulness of the microsatellites for genetic

**Table 5.** Effect of interaction of heat treatment (35 °C) and genotype on yield contributing characters.

Genotype	Treatment	NGSL	NUSL	NGP	GW	TGW	HI
Shatabdi	Control	2.957 <sup>b-e</sup>	0.680 <sup>f</sup>	263.3 <sup>a-c</sup>	11.26 <sup>b</sup>	42.90 <sup>b-d</sup>	28.60 <sup>d</sup>
	35°C	2.303 <sup>g</sup>	0.691 <sup>f</sup>	160.8 <sup>hi</sup>	6.843 <sup>gh</sup>	38.74 <sup>e</sup>	21.26 <sup>ef</sup>
BARI gom-23	Control	2.527 <sup>fg</sup>	0.692 <sup>f</sup>	176.6 <sup>gh</sup>	8.293 <sup>ef</sup>	44.10 <sup>a-c</sup>	20.83 <sup>e-g</sup>
	35°C	1.893 <sup>hi</sup>	0.801 <sup>c-e</sup>	127.2 <sup>j</sup>	4.507 <sup>jk</sup>	26.30 <sup>h</sup>	15.74 <sup>h</sup>
BARI gom-26	Control	3.357 <sup>a</sup>	0.534 <sup>hi</sup>	233.9 <sup>c-e</sup>	9.700 <sup>c</sup>	41.55 <sup>b-e</sup>	28.33 <sup>d</sup>
	35°C	1.923 <sup>hi</sup>	0.822 <sup>a-c</sup>	175.2 <sup>gh</sup>	4.130 <sup>k</sup>	25.26 <sup>h</sup>	14.87 <sup>h</sup>
BARI gom-28	Control	3.187 <sup>ab</sup>	0.639 <sup>fg</sup>	274.3 <sup>ab</sup>	11.96 <sup>ab</sup>	45.33 <sup>ab</sup>	35.04 <sup>a</sup>
	35°C	1.687 <sup>i</sup>	0.912 <sup>a</sup>	188.4 <sup>f-h</sup>	9.163 <sup>c-e</sup>	28.92 <sup>gh</sup>	33.42 <sup>ab</sup>
BARI gom-29	Control	3.100 <sup>a-c</sup>	0.712 <sup>ef</sup>	264.0 <sup>a-c</sup>	11.82 <sup>ab</sup>	47.51 <sup>a</sup>	33.33 <sup>ab</sup>
	35°C	1.987 <sup>h</sup>	0.916 <sup>a</sup>	167.6 <sup>h</sup>	9.717 <sup>c</sup>	26.94 <sup>gh</sup>	35.00 <sup>a</sup>
BARI gom-30	Control	3.197 <sup>ab</sup>	0.575 <sup>g-i</sup>	287.6 <sup>a</sup>	12.56 <sup>a</sup>	40.59 <sup>c-e</sup>	32.03 <sup>bc</sup>
	35°C	2.550 <sup>fg</sup>	0.908 <sup>ab</sup>	178.6 <sup>gh</sup>	9.457 <sup>cd</sup>	30.84 <sup>fg</sup>	32.33 <sup>bc</sup>
BARI gom-25	Control	2.560 <sup>fg</sup>	0.721 <sup>d-f</sup>	154.1 <sup>h-j</sup>	7.527 <sup>fg</sup>	43.20 <sup>bc</sup>	19.33 <sup>g</sup>
	35°C	1.850 <sup>hi</sup>	0.873 <sup>a-c</sup>	131.0 <sup>ij</sup>	5.397 <sup>ij</sup>	29.31 <sup>gh</sup>	19.54 <sup>fg</sup>
BARI gom-24	Control	3.057 <sup>bc</sup>	0.621 <sup>f-h</sup>	253.4 <sup>bc</sup>	12.48 <sup>a</sup>	48.14 <sup>a</sup>	30.67 <sup>c</sup>
	35°C	2.723 <sup>ef</sup>	0.810 <sup>b-d</sup>	239.2 <sup>cd</sup>	8.023 <sup>ef</sup>	34.36 <sup>f</sup>	27.73 <sup>d</sup>
BARI gom-20	Control	3.027 <sup>bc</sup>	0.518 <sup>i</sup>	202.9 <sup>e-g</sup>	7.357 <sup>fg</sup>	38.86 <sup>de</sup>	21.38 <sup>ef</sup>
	35°C	2.750 <sup>d-f</sup>	0.571 <sup>g-i</sup>	181.2 <sup>gh</sup>	5.983 <sup>hi</sup>	28.66 <sup>gh</sup>	19.83 <sup>fg</sup>
BARI gom-22	Control	2.997 <sup>b-d</sup>	0.566 <sup>g-i</sup>	219.4 <sup>d-f</sup>	8.417 <sup>d-f</sup>	38.66 <sup>e</sup>	22.51 <sup>e</sup>
	35°C	2.860 <sup>c-e</sup>	0.705 <sup>ef</sup>	177.2 <sup>gh</sup>	6.810 <sup>gh</sup>	28.96 <sup>gh</sup>	22.59 <sup>e</sup>
	P-value	**	**	**	**	**	**
	LSD(0.05)	0.245	0.09	30.38	1.055	3.729	1.714
	LSD(0.01)	0.328	0.121	40.65	1.412	4.99	2.294
	CV (%)	5.68	8.01	9.08	7.46	6.2	4.04

In a column, figures with same letter (s) or without letter do not differ significantly whereas figures with dissimilar letter differ significantly (as per DMRT). \*\*, \* = Significant at 1% and 5%, respectively level of probability, NGSL= No. of unfilled grain spikelet<sup>-1</sup>, NUSL= No. of unfilled grain spikelet<sup>-1</sup>, NGP= No. of grain plant<sup>-1</sup>, GW= Grain weight plant<sup>-1</sup>(g), TGW= 1000-Grain weight (g) and HI= Harvest index.

diversity and screening of heat tolerance of the 26 wheat varieties. The 7 SSRs produced a total of 44 alleles ranging from 2 to 10 with an average of 6.28 alleles per marker. Markers Xgwm577 produced the highest number of alleles (10), whereas the Xgwm428 produced the lowest number of alleles (2) (Table 7). This finding agrees with earlier results of Prasad et al., (2000) and Amer et al., (2001). Such variation in the number of allele amplified by different primer sets is attributable to several factors including primer structure and number of annealing sites in the genome (Kernodle et al., 1993). Obviously, polymorphic bands revealing differences

among genotypes would be used to examine and establish systematic relationships among genotypes as reported by Hadrys et al. (1992).

Polymorphic information content (PIC) values were estimated as a measure of genetic diversity among the genotypes. A PIC higher than 0.5, between 0.5 and 0.25 and less than 0.25, has been used as scale for loci polymorphism to be considered high, medium or low, respectively (Vaiman et al., 1994). In the current study, PIC values ranged from 0.33 for Xgwm428 to 0.87 for Xgwm577, with an average of 0.68 per marker (Table 7). Hence, the PIC values recorded in this study are high.,



**Table 6.** Effect of heat treatment on heat tolerance parameter of wheat.

Genotype	Decrease (%) (NGS)	Decrease (%) (TDM)	Decrease (%) (GW)	HTE	HSI
Shatabdi	30.91	18.394	39.225	60.775	1.269
BARI gom-23	29.75	39.744	45.640	54.360	1.476
BARI gom-26	37.79	26.619	57.429	42.571	1.858
BARI gom-28	33.03	29.634	23.393	76.607	0.757
BARI gom-29	26.76	45.205	17.790	82.210	0.575
BARI gom-30	25.06	38.276	24.715	75.285	0.799
BARI gom-25	11.69	32.744	28.292	71.708	0.915
BARI gom-24	17.56	32.363	35.724	64.276	1.156
BARI gom-20	8.47	37.028	18.640	81.360	0.603
BARI gom-22	11.62	43.710	19.049	80.951	0.616

Here, NGS= No. of grain spike<sup>-1</sup>; TDM= Total dry matter; GW= Grain weight plant<sup>-1</sup>; HTE= Heat tolerance efficiency and HSI = Heat susceptibility index.

**Table 7.** Summary statistics of 7 SSR markers found among 26 wheat genotype.

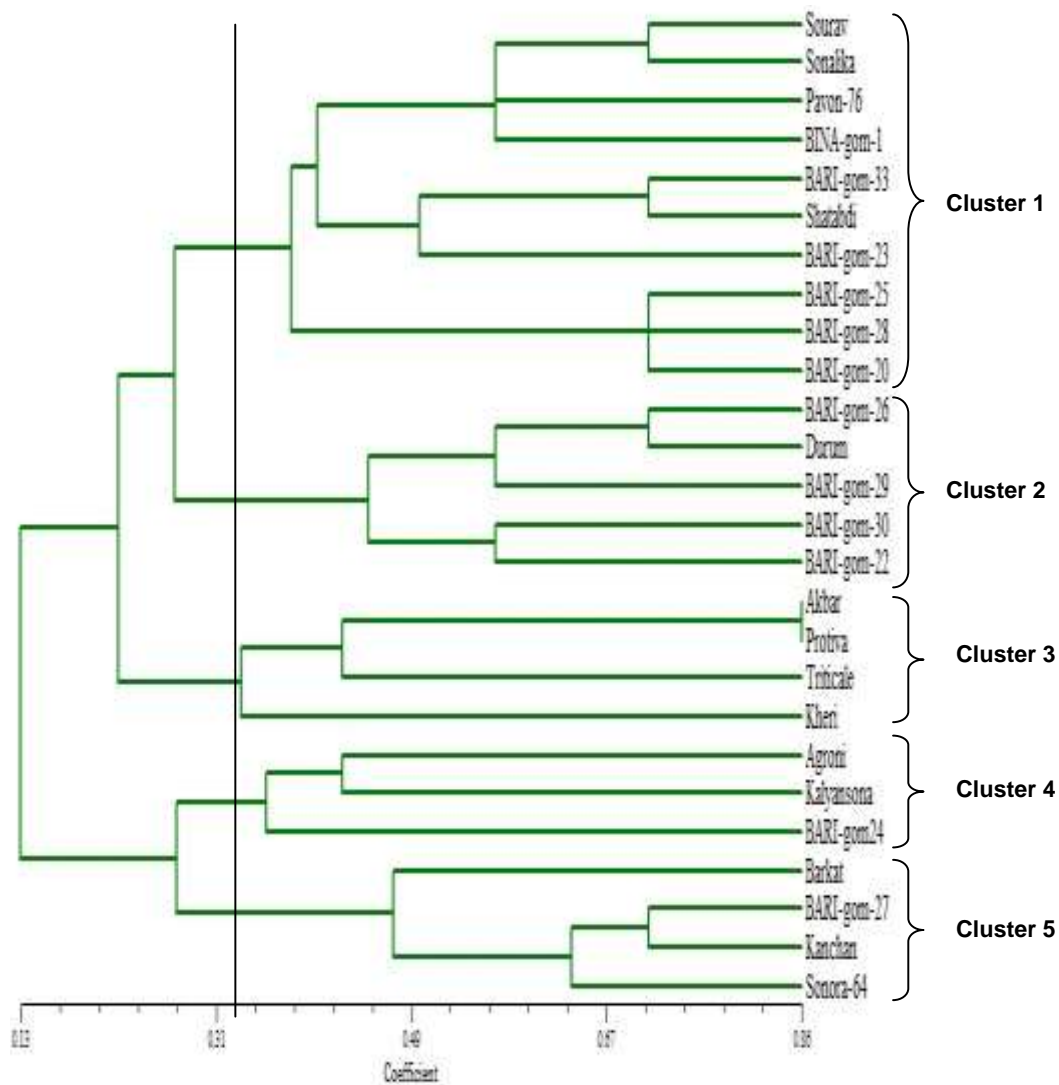
Marker name	Allele no.	Rare allele	Null allele	Major allele		Gene diversity	PIC
				Frequency	Size (bp)		
Xbarc84	7	1	0	0.2692	125	0.8107	0.7845
Xgwm132	5	1	0	0.3846	119	0.7278	0.6824
Xgwm285	8	-	0	0.1923	213	0.8609	0.8449
Xgwm428	2	-	0	0.6923	191	0.4260	0.3353
Xgwm577	10	1	0	0.1923	136	0.8817	0.8701
Xgwm617	8	1	0	0.2692	116	0.8284	0.8075
Xbarc121	4	2	2	0.5769	160	0.5444	0.4619
<b>Mean</b>	<b>6.285</b>	<b>0.857</b>	<b>0.285</b>	<b>0.3681</b>	<b>151</b>	<b>0.7257</b>	<b>0.6838</b>

and significantly higher than the PIC values reported from other studies Roder et al. (1995) and Plaschke et al (1995) but Uddin and Boerner (2008) found similar observations. The markers showed an average PIC values of 0.68 which confirm that SSR markers used in this study were highly informative because PIC values higher than 0.50 indicate high polymorphism. According to Saghai-Marouf et al., (1984), markers with PIC values of 0.5 or higher are highly informative for genetic studies. The PIC can be looked as the measurement of usefulness of each marker in distinguishing one individual from another. The PIC values and rare alleles are proved to be useful information in genetic diversity analysis of genotypes. The simple sequence repeats (SSRs) represent the most suitable marker system in wheat (Hammer et al., 2000) and have been successfully used to characterize genetic diversity in advanced wheat breeding materials Dreisigacker (2004).

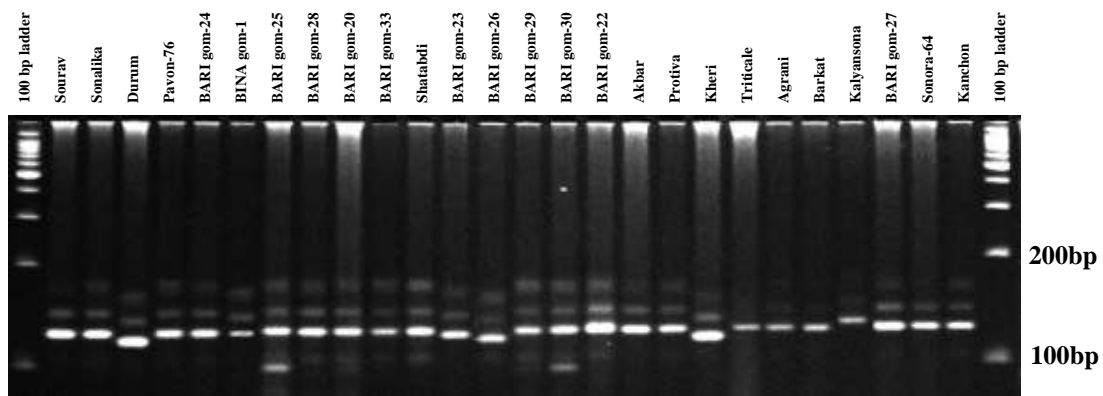
#### Genetic similarity analysis using weighted pair group method of arithmetic mean (UPGMA)

A dendrogram was constructed based on the Nei's

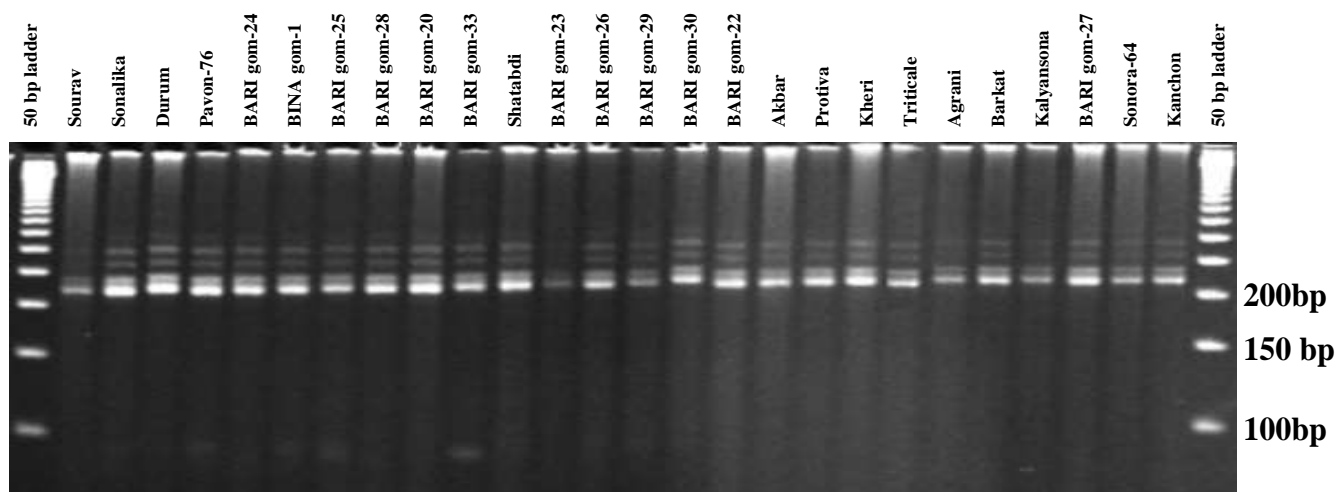
(1973) genetic distance calculated from the 44 SSR alleles (by 7 SSR Primer) generated from 26 wheat genotypes. All 26 wheat genotypes could be easily distinguished. The UPGMA cluster analysis showed significant genetic variation among the wheat genotype studied, with a similarity coefficient varying between 0.13 and 0.86. The UPGMA cluster analysis led to the grouping of the 26 germplasm into five major clusters formed at 0.33 cut off similarity coefficient below which the similarity values narrowed conspicuously. All the clusters were subdivided into two sub clusters (Figure 1). The cluster-1 consisted with ten genotypes, of which one tolerant (BARI gom-28), two moderately tolerant (BARI gom-20 and BARI gom-25) and two susceptible genotypes (BARI gom-23 and Shatabdi). Similarly the cluster-2 grouped with five genotypes, of which two tolerant (BARI gom-29 and BARI gom-30), one moderately tolerant (BARI gom-22) and one susceptible (BARI gom-26) genotype as well as another cluster-4 was contained a susceptible genotype (BARI gom-24). These clusters were contained also some genotypes which was not included in phenotypic study in our experiment. The phenotypically studied tolerant, moderately tolerant and susceptible genotypes were



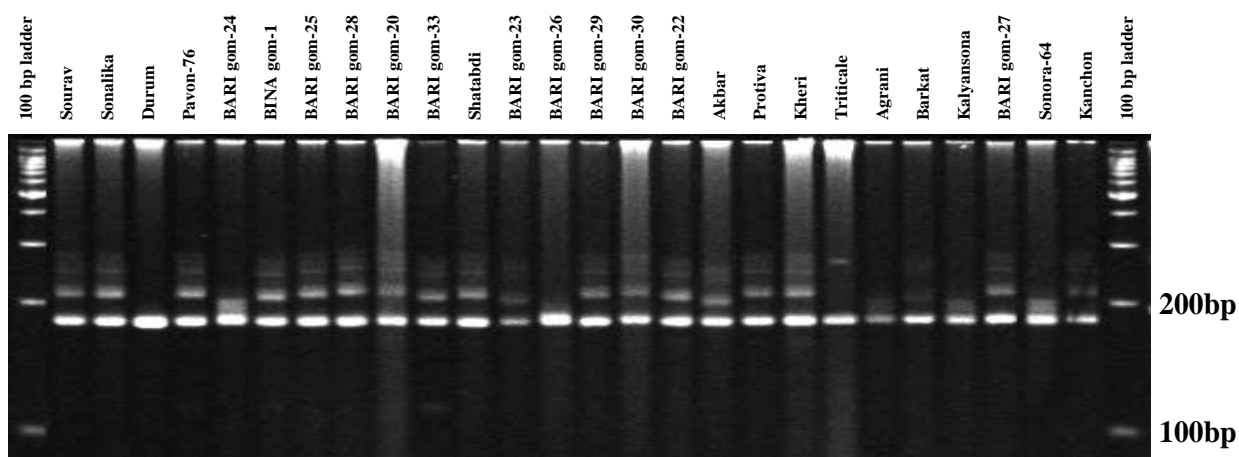
**Figure 1.** UPGMA cluster for 26 Wheat genotypes showing the genetic diversity and relatedness among them.



**Figure 2a.** Banding pattern of allele at locus Xbarc84 in 26 wheat genotypes (wells 2-27). Wells 1 and 28 are 100 bp ladders.



**Figure 2b.** Banding pattern of allele at locus Xgwm285 in 26 wheat genotypes (wells 2-27). Wells 1 and 28 represent 50 bp ladders.



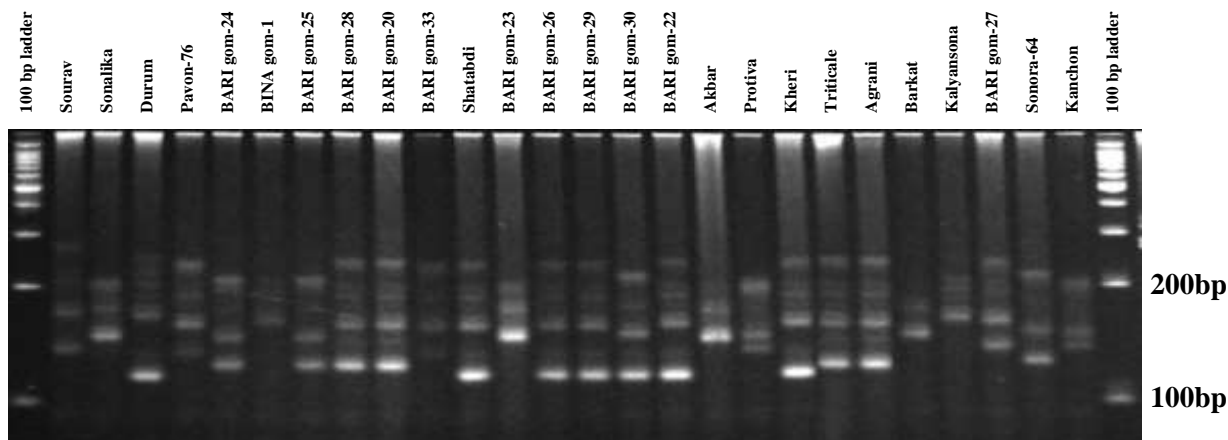
**Figure 2c.** Banding pattern of allele at locus Xgwm428 in 26 wheat genotypes (wells 2-27). Wells 1 and 28 are 100 bp ladders.

randomly present in the three clusters (1, 2 and 4). The reason for their inclusion in this same cluster is obscure. The potential of these genotypes to be tolerant to heat needs to be reevaluated in future study. Although the genotypes included in the phenotypic study have been known to be heat tolerant (DHCROP, 2018), our experiment revealed that three of them were tolerant, three were moderately tolerant and rest was susceptible. The marker assisted study revealed a discrimination of the 26 genotypes into 5 clusters. Two tolerant genotypes namely BARI gom-29 and BARI gom-30 and one moderately tolerant genotype BARI gom-22 were in cluster-2. As these three genotypes were found to be tolerant both in morphological and molecular studies of successive two years they can be recommended for

cultivation in the north-western part of Bangladesh as heat tolerant variety.

## Conclusion

The results of molecular and physiological characterization were taken under consideration simultaneously. It was observed that the genotypes showed nearly distinct arrangement according to their performance in physiological characterization. Although the genotypes included in the phenotypic study have been known to be heat tolerant, our experiment revealed that three of them were tolerant, three were moderately tolerant and rest were susceptible. The marker assisted



**Figure 2e.** Banding pattern of allele at locus Xgwm617 in 26 wheat genotypes (wells 2-27). Well 1 and 28 are 100 bp ladders.

study revealed a discrimination of the 26 genotypes into 5 clusters. Two tolerant genotypes namely BARI gom-29 and BARI gom-30 and one moderate genotype BARI gom-22 were in cluster-2. As these three genotypes were found to be tolerant both in morphological and molecular studies of successive two years they can be recommended for cultivation in the north-western part of Bangladesh as heat tolerant variety.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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