CHARACTERIZATION OF BREAD WHEAT GENOTYPES USING SSR MARKERS FOR TERMINAL HEAT TOLERANCE

Abstract

Wheat is one of the most important crops in the world. Though, wheat yield has reduced due to heat stress affectation threat to sustainability and world food security in agricultural production. The first stage of heat tolerant breeding follows on the molecular and biochemical characterization and classification of wheat genotypes. The aim of the present study is characterization of widely grown bread wheat cultivars and breeding lines for heat tolerance so as to be adapted to different regions in Bangladesh. The genotypes were screened with molecular markers for the presence of QTLs mapped to different chromosomes. Results of the molecular studies identified and detected 13 polymorphic SSR markers which gave the clearest PCR bands among the genotypes. At the end of the research, bread wheat genotypes which were classified for tolerance or sensitivity to heat and the genetic similarity within varieties were determined by molecular markers. The molecular screening with SSR primers, genetic similarity coefficients were ranged from 0.00 to 0.925. The lowest genetic distance (0.000) was found in Nadi 2 vs BAW1147 variety pair indicating that they are genetically similar to each other. Comparatively higher genetic distance (0. 925) was observed between BAW 1290 vs BARI Gom 28. The dendrogram classified fifteen genotypes into two broad groups, A and B. The two groups were generated at a similar coefficient of 0.05. Group A consisted of seven genotypes and was further subdivided into two clusters. Group B consisted of eight genotypes which were further subdivided into two clusters. The mean HSI, TGW, grain yield and relative reduction in TGW & grain yield under stress condition over timely sown condition was the basis of categorization of genotypes parallel to the molecular data. Genotypes BARI Gom 25, BARI Gom 26, BARI Gom 27, BARI Gom 28, BARI Gom 29, BARI Gom 30, and BARI Gom 31 have proved their suitability for late sown condition out of fifteen genotypes. The genotypes as heat tolerance due to their SSR markers scores are expected to provide useful information for heat related molecular breeding studies.

Keywords: *Bread wheat, SSR markers, heat tolerant, gene diversity*

Introduction

Global warming is a threat to world food security. The temperature increase has a direct impact on agricultural activities. Plant productivity is declining because of various climatic events that have increased or changed, and they threaten global food security (Mickelbart et al. 2015). When plants are exposed to abiotic stress conditions such as high temperature, drought, salinity, and excessive rainfall this affects the development and growth of the plant negatively and it causes metabolic and physiological changes in the plant. Changing climate events are predicted to cause an increase in the frequency of floods, high temperatures and drought (Bita and Gerats 2013). In these events, heat is the major abiotic stress factor that adversely affects crop production and quality especially wheat. The wheat being affected negatively from heat and climate changes makes the situation worse.

Terminal heat stress with ambient temperature > 30˚C, during reproductive development stage of wheat has been found to decrease productivity (Hays et al. 2007). Accompanied with rising mean temperature as a result of global warming, high temperature at the time of grain filling affects grain production in many environments (Hays et al. 2007). It is one of the major causes of yield reduction

which affects more than 36 million hectares in temperate environments (Reynolds et al. 2001). A significant portion of wheat grown in South Asia is considered to be affected by heat stress (Joshi et al. 2007). Bala et al. (2014) showed that high temperature significantly decreased grain yield, number of grains per kernel, plant height, grain filling duration, peduncle length, peduncle weight and 1000 kernel weight. Heat stress during grain filling is responsible for shortening of grain growth period and improper grain filling affects over-all yield of wheat crop (Rane et al. 2007).

Most crops, increased in yield by conventional breeding methods, grow under favorable conditions. For this reason most of the varieties of crops used for agricultural production are not tolerant to heat. Therefore, it is very important that biotechnological studies continue at a rapid speed in order to develop heat-tolerant plants that can adapt to local conditions, since they have a large share in production. The first stage of heat tolerant variety breeding begins with research studies focusing on various aspects such as reducing the effects of heat, molecular and biochemical characterization and classification of wheat varieties and putting them in terms of tolerance to heat. Bangladesh produce ten lakh tons of wheat in year. Despite of its importance in the world production, some regions in Bangladesh are at the risk of heat that is caused by the effects of global climate change.

Wheat has a large genome sizes (16.000 Mb for bread wheat), therefore, heat tolerance is a complex and quantitative trait controlled by multiple genes. In order to overcome these challenges, many studies have been carried out about the molecular mechanism of heat tolerance and molecular breeding for heat. Recently, several molecular markers and quantitative trait loci (QTLs) have been found to be associated with genes responsible for the heat signaling mechanism. Significant progress has been made in molecular identification of genes of interest (Yıldırım et al. 2013). These improvements have allowed for the development of crop that is tolerant to heat conditions in future. To achieve this, numerous molecular markers are used. Among these markers, the most notable ones are the DNA markers which are based on the Polymerase Chain Reaction (PCR). In genetic characterization studies of wheat; amplified fragment length polymorphisms (AFLP) (Barrett and Kidwell 1998), Sequence tagged microsatellite site markers (STMSs) or generally simple sequence repeats (SSRs) (Prasad et al. 2000) and chloroplast-specific microsatellite markers (CPSSR) (Tomar et al. 2013) are all used as molecular markers based on PCR. Golabadi et al. (2011) used microsatellite markers to identify QTLs with yieldtrait competent such as thousand grain weight and harvest index. In another study, Ramya et al. (2015) reported about physiological and genetic characterization of 24 modern wheat genotypes to use in breeding studies to examine the drought and temperature tolerance. Taking into account the results from previous studies, it can be concluded that SSR markers can be effectively used to determinate heat tolerance in wheat.

The high yielding wheat genotypes under heat stress can be identified by calculating Heat susceptibility index (HSI) following field evaluation for a number of agronomic traits (Kirigwi et al. 2007; Mohammadi et al. 2008; Mason et al. 2010, 2011). Characterization of wheat genotypes for high temperature tolerance identified genotypes with better relative performance in yield components, grain yield and heat susceptibility index (Rahman et al. 2009; Khan et al. 2014). The use of HSI and performance under late sowing heat stressed conditions has also been reported in a number of studies earlier as well (Mohammadi et al. 2008; Pinto et al. 2010; Yang et al. 2010; Barakat et al. 2011; Mason et al. 2010, 2011). However, in order to conduct genetic analyses in the form of QTL studies, the genotypes with contrasting characters needs to identified. The aim of this study is to characterization of bread wheat genotypes for heat tolerance and that has adapted to different local conditions of Bangladesh.

Materials and methods

Plant material

Thirteen bread wheat cultivars (Triticum aestivum L.) and 2 breeding lines were used to assessing the molecular diversity for terminal heat stress tolerance against 13 SSR markers linked to the trait of interest (Sadat et al., 2013). All the genotypes were collected from Bangladesh Wheat and Maize Agricultural Research Institute (BWMRI).The genotypes were evaluated under field conditions at Regional Station (RS), BWMRI, Gazipur during rabi 2021-22.The lab experiment was conducted in Molecular Laboratory, RS, BWMRI, Gazipur. Pedigree of the bread wheat genotypes are summarized in Table 1.

Table 1. List of fifteen wheat genotypes with their pedigree

Variety		Year of release	Life cycle (days)	Special features
BARI Gom 25	ZSH 12/HLB 19//2*NL297	2010	102-110	• Heat and salinity tolerant. • It can tolerate 8-10dS/m salinity at seedling stage. • High yielding (3.8-5.0 t/ha) • Resistant to leaf rust and Leaf blight 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 tolerant. • Suitable for late sowing • High yielding (4.0-5.0 t/ha) • Resistant to leaf and stem rust (Ug99 race) and moderately resistant to leaf blight
BARI Gom 27	WAXWING*2/VIVISTI CGSS01BOOO56T-099Y-099M-099M-099Y-099M-14Y-0B	2012	105-110	• High yielding (3.5-5.4 t/ha) • Resistant to leaf and stem rust (Ug99 race) and moderately resistant to leaf blight
BARI Gom 28	CHIL/2*STAR/4/BOW/CROW//BUC/PVN/3/2*VEE#10 CMSS95Y00624S-0100Y-0200M-17Y-010M-5Y-0M	2012	102-108	• Early maturing and heat tolerant. • Suitable for late sowing • High yielding (4.0-5.5 t/ha) • Resistant to leaf and moderately resistant to leaf blight
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	• Short stature, tolerant to lodging. • Moderately tolerant to heat stress • High yielding (4.0-5.0 t/ha) • Resistant to leaf and stem rust (Ug99 race) and moderately resistant to leaf blight
BARI Gom 30	BAW 677/Bijoy BD(JA)1365S-0DI-15DI-3DI-HR12R3DI	2014	100-105	• Early maturing and heat tolerant. • Suitable for late sowing • High yielding (4.0-5.5 t/ha) • Resistant to leaf and moderately resistant to leaf blight
BARI Gom 31	KAL/BB/YD/3/PASTOR CMSS99M00981S-0P0M-040SY-040M-040SY-16M-0ZTY-0M…	2017	104-109	• Early maturing, heat tolerant • Resistant to LR and tolerant to spot blotch • Yield:4.5-5.0 t/ha
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
BARI Gom 33		2018		•
WMRI Gom 1		2019		•
WMRI Gom 2				•
WMRI Gom 3				•
BAW 1290				•
BAW 1147				•
Nadi 2				•

Extraction of DNA and SSR analysis

Genomic DNA of 15 wheat genotypes was extracted from fresh leaves. Total genomic deoxyribonucleic acid (DNA) from individual genotype was extracted from young leaves at seedling stage by CTAB (cetyltrimethtyl ammonium bromide) method with some modified. Thirteen SSR markers (gwm291, Gwm325, Xgwm294, Gwm268, Xwmc407, Xcfa2129, gwm11, Xcfd43, Xgwm356, Xbarc137, Gwm484, Gwm293, WMC527) were selected from different location of chromosomes (Table 2).

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

S. N.	Marker	**QTL for**	Primers sequence Reverse (5'-3')	Primers sequence Forward (5'-3')	Chromosomal location	Annealing temp. (˚C
1	gwm291	Leaf Curl	AATGGTATCTATTCCGACCCG	CATCCCTAGGCCACTCTGC	5A	60
2	Gwm325	HSI grain filling duration HSI kernel weight	TTTTTACGCGTCAACGACG	TTTCTTCTGTCGTTCTCTTCCC	6D	60
3	Xgwm294	HSI_single kernel weight of main spike	GCAGAGTGATCAATGCCAGA	GGATTGGAGTTAAGAGAGAACCG	2A	55
4	Gwm268	HSI kernel weight	TTATGTGATTGCGTACGTACCC	AGGGGATATGTTGTCACTCCA	1B	55
5	Xwmc407	Grain-filling duration	CATATTTCCAAATCCCCAACTC	GGTAATTCTAGGCTGACATATGCTC	2A	61
6	Xcfa2129	HSI_single kernel weight of main spike	ATCGCTCACTCACTATCGGG	GTTGCACGACCTACAAAGCA	1A, 1B, 1D	60
7	gwm11	Grain-filling duration	GTGAATTGTGTCTTGTATGCTTCC	GGATAGTCAGACAATTCTTGTG	1A, 1B	50
8	Xcfd43	Grain-filling duration	CCAAAAACATGGTTAAAGGGG	AACAAAAGTCGGTGCAGTCC	2D	60
9	Xgwm356	HSI single kernel weight of main spike	CCAATCAGCCTGCAACAAC	AGCGTTCTTGGGAATTAGAGA	2A, 6A, 7A	55
10	Xbarc137	Waxiness	CCAGCCCCTCTACACATTTT	GGCCCATTTCCCACTTTCCA	1B	52
11	Gwm484	Waxiness	AGTTCCGGTCATGGCTAGG	ACATCGCTCTTCACAAACCC	2D	55
12	Gwm293	Grain-filling duration	TCGCCATCACTCGTTCAAG	TACTGGTTCACATTGGTGCG	5A	55
13	WMC527	HSI_kernel weight of main spike	GCTACAGAAAACCGGAGCCTAT	ACCCAAGATTGGTGGCAGAA	3A, 3B	61

Fresh leaves of each genotype were ground in mortar with a pestle and transferred to a 2 ml centrifuge tube. Chloroform (400 µl) was added and mixed gently by inverting the tube and heat in a water bath at 65ºC for 1 hour. Then centrifuge the sample at 12000 rpm for 10 minutes at 4ºC. The supernatant was collected into a new 1.5 ml tube. Equal volume of isopropyl alcohol (Isopropanol) was added and mix gently by inverting (2-3 times). The samples were kept for 2 hours at -20 ºC for precipitating the DNA. Centrifuge the samples at 12000 rpm for 15 min at 4ºC. A very small gel like

pellet should be visible at the bottom of the tube. Centrifuge the pellet with 0.4 ml (400 µl) of 75% chilled ethanol for 5-8 minutes with 6000-8000 rpm (for 2 times). The final pellets were air dried for 24 hrs. The pellets were dissolved in 100 µl of 1X TE buffer. A spectrophotometer was used to check the concentration and quality of extracted DNA for the Polymerase Chain Reaction (PCR) amplification. Thirteen primer pairs were used for the analysis of SSR. PCR conditions were maintained as described by Roder [27].

Each PCR was carried out in a about 10 µl reaction volume containing Nuclease free water, Master mix, and both primer pairs according to the primers profile. The PCR amplification of wheat genomic DNA was done by incubating the DNA samples for 5 minutes at 94°C, then 45 cycles comprising 94°C for 60 seconds, annealing of primer for 60 seconds at 58-60°C and the extension for 60 seconds at 72°C. The final extension was carried out for 10 minutes at 72°C.

The PCR products were electrophorized on 1.5% of agrose gel containing 10 µl ethidium bromide, at 100 volts for 25 minutes using horizontal gel electrophoresis assembly. After 75% of the gel run, the amplicons were visualized and photographed under UV light (Cleaver Scientific Ltd., UK).

Statistical analysis

Every band was considered as a single locus. All the scorable Loci were considered for generation of bivariate 1-0 data matrix and genetic distances (GD) among the genotypes were estimated using Unwaited Pair Group of Arthematic Means (UPGMA) as described by share allele and for estimation of genetic diversity, dendrogram, Polymorphism information content (PIC) value was constructed using the software Power marker.

Heat Susceptibility Index (HSI)

Heat susceptibility index (HSI) was used to evaluate the effect of heat stress on thousand grain weight (TGW) & grain yield. The formula used for HSI calculation, taken from Paliwal et al. (2012), is given below:

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

Where,

X represents TGW & Grain yield

X_{heat} stress represents phenotypic values of individual genotypes for TGW & Grain yield under late sowing

$X_{control}$ represents phenotypic values of individual genotypes for TGW & Grain yield under normal sowing

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

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

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

Results and Discussion

A total of 13 SSR primers were used in this study (Table 2; Fig. 1). The allele numbers and allele sizes of the primers are presented in Table 3. The number of alleles detected by the primers ranged from 2 to 8 among the bread wheat genotypes. The most polymorphic microsatellite marker was Gwm293 with 8 alleles, followed by Xgwm356 and had 7 alleles (Table 3). A total 51 polymorphic allele were obtained from screened 15 bread wheat genotypes using the 13 SSR markers with an average of 3.92 alleles per locus. The lowest number of alleles was found in Xcfd43 with 2 alleles.

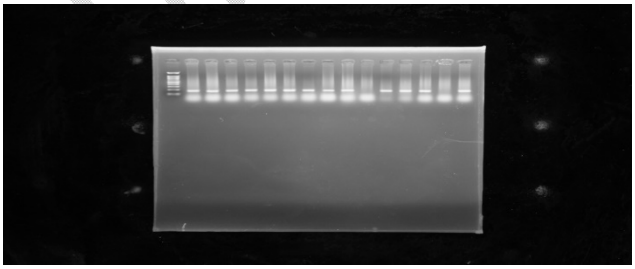
The polymorphism information content (PIC) values of the analyzed microsatellite markers ranged from 0.325 (Xwmc407) to 0.827 (Xgwm356) with a mean PIC value being 0.567. This is close to the result revealed by Zheng et al. (2009). Among the 13 SSR markers used in this study, Xgwm356 primer had the

highest PIC values (0.827), followed by primer Gwm293 with 0.818 PIC value. According to PIC values of each marker; the lowest PIC value was presenting Xwmc407 primer with 0.325 PIC. It was found that the highest heterozygosity (He) value was found in Xgwm294, Xcfa2129, Gwm293primer with the value of 1.00 and the lowest 0.325 (Xwmc407) to 0.827 (Xgwm356) with a mean PIC value being 0.567. He value was found in gwm291, Gwm325, Xwmc407, gwm11, Xcfd43, Xbarc137 primer with 0.0 value. The most widely used principle for testing genetic variation in a population is heterozygosity.

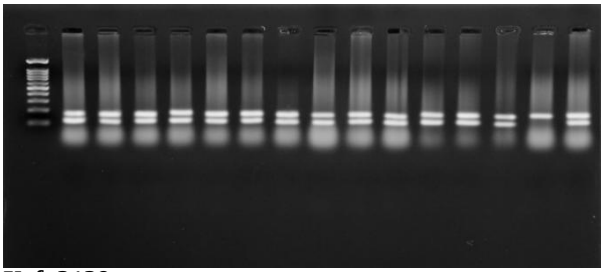
The SSR markers used in this study indicates low heterozygosity with an average of 0.372 He value in the investigated wheat genotypes have narrow genetic variation. PIC value for the primers used in this study, had 13 loci that were considered to be informative, since they had a PIC value greater than 0.5. The PIC value can be used to evaluate the level of gene variation in a plant. When the PIC value is >0.5, the locus is considered to be of high diversity, while if the PIC is <0.25 the locus is considered to be of low diversity (Botstein et al. 1980; Nagy et al. 2012; Ramadugu et al. 2015). The mean PIC value used SSR markers in this study was 0.567 and the range was from 0.325 to 0.827.

Table 3. Allele numbers and sizes, PIC value found in 15 wheat genotypes for 13 SSR markers.

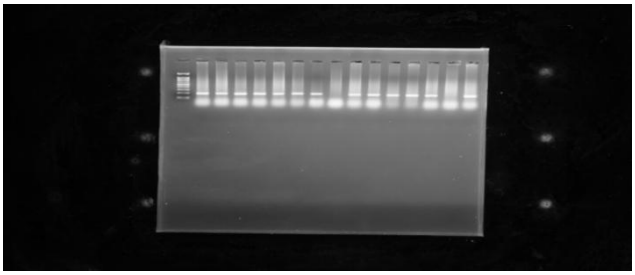
Marker	Allele No	Allele size and range	Difference (bp)	Major.Allele Frquency	Gene Diversity	Heterozygosity (He)	PIC
gwm291	3	150-160	10	0.4615	0.639	0.000	0.566
Gwm325	3	150-160	10	0.3750	0.656	0.000	0.582
Xgwm294	4	50-120	70	0.5000	0.645	1.000	0.587
Gwm268	3	180-285	105	0.6667	0.475	0.111	0.404
Xwmc407	2	140-145	5	0.7143	0.408	0.000	0.325
Xcfa2129	4	120-190	70	0.4667	0.656	1.000	0.592
gwm11	3	200-210	10	0.7143	0.439	0.000	0.386
Xcfd43	2	160-165	5	0.5000	0.500	0.000	0.375
Xgwm356	7	185-230	45	0.2000	0.847	0.800	0.827
Xbarc137	4	245-260	15	0.4444	0.667	0.000	0.607
Gwm484	4	90-190	100	0.3462	0.710	0.923	0.656
Gwm293	8	105-190	85	0.2333	0.838	1.000	0.818
WMC527	4	345-450	105	0.4000	0.700	0.000	0.645
Mean	**3.92**			**0.4633**	**0.629**	**0.372**	**0.567**
Total	**51**						
Range	**2-8**		**2-105**	**0.2000-0.7143**	**0.439-0.847**	**0-1**	**0.325 - 0.827**



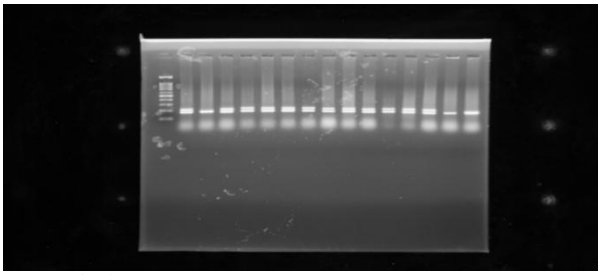
gwm291



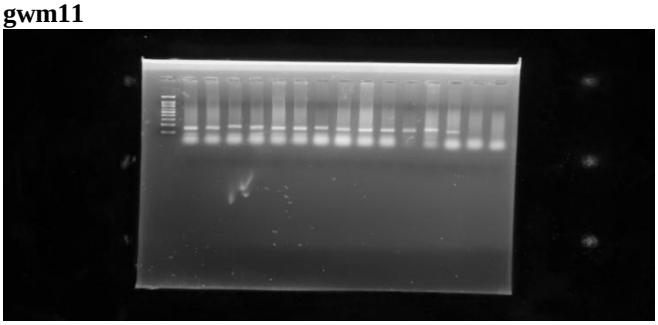
Xcfa2129



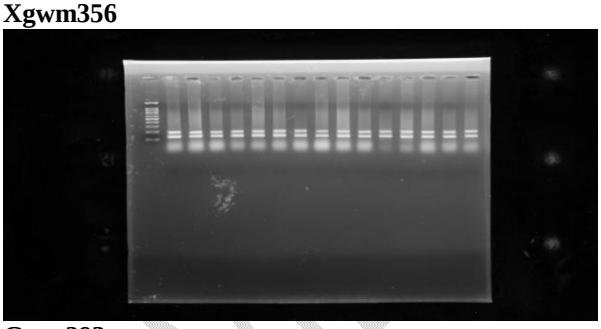
gwm11



Xgwm356



Gwm484



Gwm293

Fig. 1 The SSR marker profile of bread wheat genotypes wheat using gwm291, Xcfa2129, gwm11, Xgwm356, Gwm484, and Gwm293 SSR primers.

Table 4. Genetic Distance of 15 wheat genotypes based on 13 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**	**Nadi 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

Therefore, most of the primers used in this study were found to be highly informative. The results that used SSRs are potential markers that could be used as marker to assist in selection for terminal heat stress tolerance by molecular plant breeding.Hao et al. (2006) suggested that the allele's number in each locus and the calculated PIC values of these alleles should be evaluated together as part of an objective assessment of genetic diversity in genotype collections. Since PIC values correlates positively with the number of alleles for all genotypes. PIC also showed a significant, positive correlation with the number of alleles range for microsatellites evaluated in this study.

The highest level of genetic diversity value (0.847) was observed in loci Xgwm356 and the lowest level of genetic diversity value (0.439) was observed in loci gwm11with a mean diversity of 0.629 (Table 3). It was observed that marker detecting the lower number of alleles showed lower gene diversity than those detected higher number of alleles which revealed higher gene diversity. This result is in consistent with previous work done by Herrera et al. (2008), who also observed that the genetic diversity at each SSR locus was significantly correlated with the number of alleles detected of microsatellite markers.

The values of pair-wise comparison of share allele, genetic distance (D) between varieties were computed from combined data for the 13 primers, ranged from 0.00 to 0.925 (Table 4). Comparatively higher genetic distance (0. 925) was observed between BAW 1290 vs BARI Gom 28 followed by WMRI Gom 3 vs BARI Gom 26 (0.889), BAW1147 vs BARI Gom 28, BARI Gom 29, BARI Gom 30, BARI Gom 31 (0.875), WMRI 1 vs BAW 1147 (0.875), WMRI Gom 2 vs BAW 1147 (0.875). The higher genetic distance between them indicates that genetically they are diverse compare to lower genetic distance value. Basically this value is an indication of their genetic dissimilarity. Variety pair with higher value is more dissimilar than a pair with a lower value. The lowest genetic distance (0.000) was found in Nadi 2 vs BAW1147 variety pair indicating that they are genetically similar to each other.

A dendrogram was constructed based on the share allele genetic distance calculated from 51 alleles generated from 15 wheat varieties. All 15 wheat cultivars could be easily distinguished. The UPGMA cluster tree analysis led to the grouping of the 15 wheat varieties in four major clusters (Fig. 2). The dendrogram classified fifteen lines into two broad groups, A and B. The two groups were generated at a distance coefficient of 0.05. Group A consisted of seven genotypes and was further subdivided into two clusters. Group B consisted of eight genotypes and was further subdivided into two clusters. Cluster wise mean values of heat susceptibility index (HSI) for TGW & grain yield, TGW & grain yield in Optimum and late sown condition and mean percentage decrease or increase in TGW & grain yield in late sown over timely sown condition as well as the values of same parameters for each member of the cluster are presented in Table 5 & 6. The two major groups obtained in cluster analysis differed with respect to three parameters as a measure of heat tolerance at field level viz., HSI, TGW and grain yield in stress condition, percent decrease in TGW and grain yield in stress over normal condition as evident from the table 5 & 6.

Heat Susceptibility Index (HSI) was measured for thousand grain weight (TGW) and grain yield to identify heat tolerant and susceptible genotypes. HSI estimates for all genotypes showed both resistant and tolerant genotypes. The HSI for TGW ranged from 0.772 to 1.218 and grain yield range from 0.742 to 1.253. These values were used for identifying heat tolerant genotypes. Low HSI (HSI<1) is synonymous with high stress tolerance (Fischer and Maurer 1978). Based upon the value and direction of desirability, different genotypes were ranked as highly heat tolerant (HSI<0.50), moderately heat tolerant (HSI 0.50-1.00) and heat susceptible (HSI>1.00) (Khanna-Chopra and Viswanathan 1999 and Singh et al. 2011).

Cluster I consisted of three genotypes namely BARI Gom 25, BARI Gom 26, and BARI Gom 27, having HSI value for thousand grain weight (TGW) and grain yield per plot in range of 0.870- 0.887 and 0.903- 0.964 respectively. These genotypes were moderately heat tolerant (HSI 0.50-1.00).Thousand grain weight (TGW) and grain yield per plot in stress condition (E2) in range of 38.45 g - 43.87g and 1.85 kg - 2.31kg in order. These genotypes suffered 10.27 % - 10.47 % decrease in TGW and 13.75 % - 14.75

% decrease in grain yield under E2 condition in comparison to that in normal environment (E1). The cluster means of HSI for TGW and grain yield and percent decrease of TGW and grain yield were 0.887, 0.903, 10.47%, and 13.81% respectively. These genotypes are close to the members of cluster II of group A.

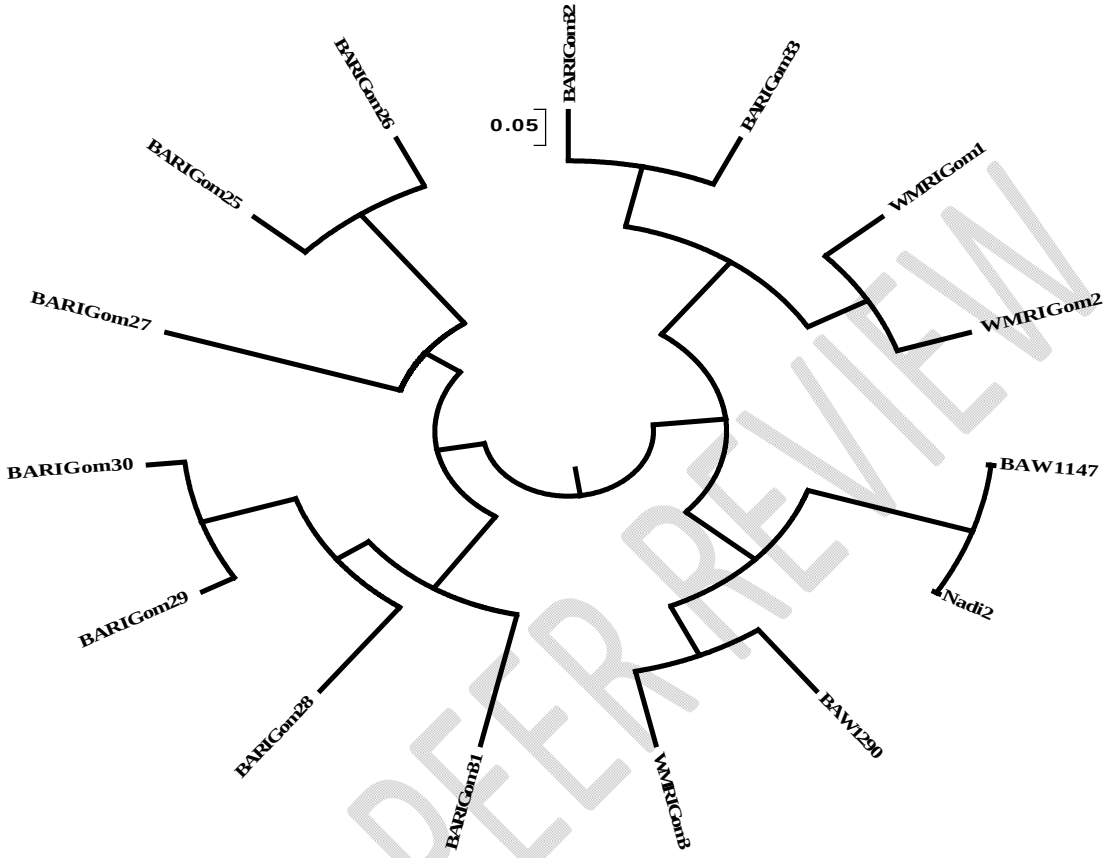


Fig.2. Dendrogram generated through UPGMA analysis showing genetic relationship among the 15 wheat genotypes. Names of the genotypes are given on the ends of branches.

Cluster II consisted of four genotypes namely BARI Gom 28, BARI Gom 29, BARI Gom 30 and BARI Gom 31. HSI for TGW (g) and grain yield/plot (kg), TGW (g) and grain yield/plot (kg) in stress condition (E2), reduction in TGW and yield compared to the normal unstressed condition (%) was observed in range of 0.772-0.909, 0.742-0.930, 37.89-42.25g, 2.11-2.37kg, 9.11-10.74%, and 11.34-14.23% in order. The cluster means of HSI for TGW and grain yield and percent decrease of TGW and grain yield were 0.772, 0.742, 9.11%, and 11.34% respectively. These genotypes were also moderately heat tolerant (HSI 0.50-1.00).

Group B consisted of eight genotypes which were further subdivided into two clusters (cluster III and IV). Cluster III comprised of four genotypes viz. WMRI 3, BAW 1290, BAW 1147, and Nadi 2. The mean HSI for TGW (g) and grain yield/plot (kg), TGW (g) and grain yield/plot (kg) in stress condition, relative reduction in TGW and grain yield under stress condition for this cluster was observed to be 1.038-1.218, 1.128-1.253, 37.86-38.57g, 1.63-2.19kg, 12.26-14.38% and 17.26-19.17% respectively. The cluster means of HSI for TGW and grain yield and percent decrease of TGW and grain yield were 1.038, 1.128, 12.26% and 17.26% respectively. These genotypes were heat susceptible (HSI>1.00).

Cluster IV consisted of four genotypes viz. BARI Gom 32, BARI Gom 33, WMRI Gom 1, and WMRI Gom 2. The mean HSI for TGW (g) and grain yield/plot (kg), TGW (g) and grain yield/plot (kg) in stress condition, relative reduction in TGW and grain yield under stress condition for this cluster was

observed to be 1.043-1.169, 1.004-1.102, 41.82-43.23g, 2.09-2.18kg, 12.31-13.80%, and 15.35-16.86% respectively. These genotypes were also heat susceptible (HSI>1.00).The cluster means of HSI for TGW and grain yield and percent decrease of TGW and grain yield were 1.169, 1.050, 13.80%, and 16.06% respectively. The results were in agreement with the results of Ali et al., (2013), Pinto et al., (2010) and Sadat et al., (2013) who used SSR markers for assessing the genetic diversity for heat stress tolerance in wheat.

Table 5. Summary of wheat genotypes clusters using morpho - physiological traits (TGW).

Cluster	Genotypes	HSI	TGW		%TGW decrease	HSI	TGW		%TGW decrease
			ITS	ILS			ITS	ILS	
Group A									
Cluster I	BARI Gom25	0.887	49.00	43.87	10.47	0.887	49.00	43.87	10.47
Cluster I	BARI Gom26	0.885	48.05	43.03	10.45				
Cluster I	BARI Gom27	0.870	42.85	38.45	10.27				
Cluster II	BARI Gom28	0.772	45.00	40.90	9.11	0.772	45.00	40.90	9.11
Cluster II	BARI Gom29	0.892	42.35	37.89	10.53				
Cluster II	BARI Gom30	0.823	46.80	42.25	9.72				
Cluster II	BARI Gom31	0.909	42.75	38.16	10.74				
Group B									
Cluster III	WMRI Gom 3	1.038	43.15	37.86	12.26	1.038	43.15	37.86	12.26
Cluster III	BAW 1290	1.181	44.30	38.12	13.95				
Cluster III	BAW 1147	1.218	45.05	38.57	14.38				
Cluster III	Nadi 2	1.106	43.55	37.86	13.07				
Cluster IV	BARI Gom32	1.169	48.55	41.85	13.80	1.169	48.55	41.85	13.80
Cluster IV	BARI Gom33	1.061	48.85	42.73	12.53				
Cluster IV	WMRI Gom 1	1.129	48.85	42.34	13.33				
Cluster IV	WMRI Gom 2	1.043	49.30	43.23	12.31				

Table 6. Summary of wheat genotypes clusters using morpho - physiological traits (Grain yield).

Cluster	Genotypes	HIS	Yld		% Yld decrease	HSI	Yld		% Yld decrease
			ITS	ILS			ITS	ILS	
Group A									
Cluster I	BARI sGom25	0.903	2.68	2.31	13.81	0.903	2.68	2.31	13.81
Cluster I	BARI Gom26	0.964	2.17	1.85	14.75				
Cluster I	BARI Gom27	0.960	2.52	2.15	14.68				
Cluster II	BARI Gom28	0.742	2.38	2.11	11.34	0.742	2.38	2.11	11.34
Cluster II	BARI Gom29	0.833	2.59	2.26	12.74				
Cluster II	BARI Gom30	0.756	2.68	2.37	11.57				
Cluster II	BARI Gom31	0.930	2.67	2.29	14.23				
Group B									
Cluster III	WMRI Gom 3	1.128	1.97	1.63	17.26	1.128	1.97	1.63	17.26
Cluster III	BAW 1290	1.189	2.64	2.16	18.18				
Cluster III	BAW 1147	1.253	2.66	2.15	19.17				

Cluster III	Nadi 2	1.135	2.65	2.19	17.36				
Cluster IV	BARI Gom32	1.050	2.49	2.09	16.06	1.050	2.49	2.09	16.06
Cluster IV	BARI Gom33	1.004	2.54	2.15	15.35				
Cluster IV	WMRI Gom 1	1.102	2.61	2.17	16.86				
Cluster IV	WMRI Gom 2	1.056	2.60	2.18	16.15				

At a glance of all the four clusters showed that cluster IV had highest mean HSI value as well as highest decrease in TGW under late sown condition over timely sown condition. But cluster III had highest mean HSI value as well as highest decrease in grain yield under late sown condition over timely sown. Though, BARI Gom 25 and BARI Gom 30 has genetic potential for higher yield as evident from its higher yield under stress as compared to other genotypes of group A. The molecular grouping of BAW 1147 as heat sensitive genotype is justified by higher HSI value for grain yield. Genotypes, BARI Gom 25, BARI Gom 28, BARI Gom 29, BARI Gom 30, and BARI Gom 31of group A have proved their suitability for late sown condition. As a consequence, morphological data of most of the genotypes supported the findings at the molecular level.

Though, some difference were detected in case of Nodi 2 of group B displayed higher reduction in mean grain yield (17.36%) in late sown condition over timely sown condition with higher HSI (1.135) value but identified highest grain yield (2.19 kg) under late sown among heat sensitive group, which is honestly symbolic of rejection from heat sensitive group. On the other hand BARI Gom 26 of group A has higher HSI value (0.964) with highest decrease in mean grain yield (14.75%) in late sown condition over timely sown condition and lowest grain yield (1.85 kg) under late sown among heat tolerant group which was similar grain yield (1.63 kg) from WMRI Gom 3 of group B, that are fairly indicative of elimination from terminal heat stress tolerant group. The reason for these dissimilarities may be that the heat stress is a regional problem. In some areas it shocks the plant for just a few hours and in other areas the stress is prolonged and spans from reproductive stage until the wheat ripens. Also, as heat stress is a complex trait that further associations with another complex trait, yield, resulting genotype × environment interaction has a thoughtful impact on the expression of yield trait. Subsequently, the assessment of the genotypes were conducted under field condition, the weather variation was obvious. The similar results were reported by Pandey et al., (2013).

The heat tolerant wheat variety is emerging priorities of agricultural research, because above the optimum temperature (21.3±1.27˚C) during reproductive stage viz. grain filling duration, wheat yield is significantly affected. So, there is a dire need to develop/identify genotypes that are either tolerant to terminal heat stress or that mature early without significant yield losses.

Molecular and genetic approaches of this study the DNA polymorphism conferring thermo tolerance will not only facilitate marker supported breeding for heat tolerance but also cover the way for cloning and characterization of fundamental genetic factors which could be useful for engineering plants for improved heat tolerance. Thus, in a nutshell, in the present investigation, the SSR markers used, proved their worthiness in categorization of the wheat genotypes as terminal heat stress susceptible or tolerant except for fewer anomaly.

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