Journal of Experimental Agriculture International

Volume 46, Issue 10, Page 565-577, 2024; Article no.JEAI.125288
ISSN: 2457-0591
(Past name: American Journal of Experimental Agriculture, Past ISSN: 2231-0606)

Characterization of Bread Wheat Genotypes Using SSR Markers for Terminal Heat Tolerance

Mohammad Forhad Amin [a], Taslima Jahan [b], Muhammad Rezaul Kabir [c], Md. Mahbubur Rahman [a], Md. Mustafa Khan [a], Md. Abdul Hakim [c] and Golam Faruq [c*]

[a] Regional Station, Bangladesh Wheat and Maize Research Institute, Gazipur, Bangladesh.
[b] Bangladesh Agricultural Research Instituite, Joydebpur, Gazipur, Bangladesh.
[c] Bangladesh Wheat and Maize Research Institute, Dinajpur, Bangladesh.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/jeai/2024/v46i102979

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/125288

Original Research Article

Received: 13/08/2024
Accepted: 15/10/2024
Published: 19/10/2024

ABSTRACT

Wheat is a major global food crop, but its productivity is increasingly threatened by terminal heat stress due to climate change. This study aimed to characterize the genetic diversity and heat tolerance of widely grown bread wheat genotypes using SSR markers to identify heat-tolerant cultivars adaptable to various regions in Bangladesh. A total of 15 genotypes were screened, and 13 polymorphic SSR (Simple Sequence Repeats) markers were used to determine the genetic similarity and categorize genotypes based on their heat tolerance. The molecular analysis revealed 13 polymorphic SSR markers that produced distinct PCR (Polymerase Chain Reaction) bands

---

**Corresponding author: E-mail: faruqwrc@gmail.com;*

***Cite as:** Amin, Mohammad Forhad, Taslima Jahan, Muhammad Rezaul Kabir, Md. Mahbubur Rahman, Md. Mustafa Khan, Md. Abdul Hakim, and Golam Faruq. 2024. "Characterization of Bread Wheat Genotypes Using SSR Markers for Terminal Heat Tolerance". Journal of Experimental Agriculture International 46 (10):565-77. https://doi.org/10.9734/jeai/2024/v46i102979.*

across the different genotypes. By the conclusion of the study, bread wheat genotypes were categorized as either tolerant or sensitive to heat, and the genetic similarity among the varieties was assessed using molecular markers. The genetic similarity coefficients obtained from the SSR primer screening 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 divided the fifteen genotypes into two main groups, A and B, both formed at a similar coefficient of 0.05. Group A included seven genotypes and was further divided into two clusters, while Group B comprised eight genotypes, which were also divided into two clusters. The categorization of the genotypes was based on the average Heat Susceptibility Index (HSI), Thousand Grain Weight (TGW), grain yield, and the relative reduction in TGW and grain yield under stress conditions compared to timely sown conditions, aligning with the molecular data. Among the fifteen genotypes, BARI Gom 25, BARI Gom 26, BARI Gom 27, BARI Gom 28, BARI Gom 29, BARI Gom 30, and BARI Gom 31 demonstrated their adaptability to late sown conditions. The heat tolerance observed in these genotypes, as indicated by their SSR marker scores, is anticipated to provide valuable insights for molecular breeding studies focused on heat resistance.

Keywords: Bread wheat; SSR markers; heat tolerant; gene diversity.

1. INTRODUCTION

Global warming presents a serious risk to global food security, as rising temperatures have a direct effect on agricultural processes. A number of climate-related changes are causing a decline in plant productivity, endangering the stability of the global food supply [1]. Plant growth and development are disrupted by exposure to abiotic stresses such as high temperatures, drought, salinity, and heavy rainfall, which result in notable physiological and metabolic alterations. According to predictions, the frequency of heat waves, droughts, and floods is expected to increase due to climate change [2]. Among these, heat is a significant stressor that has a detrimental effect on crop quality and productivity, particularly in wheat, making the problems caused by changing climate patterns worse.

Terminal heat stress, when temperatures rise above 30°C during the reproductive phase of wheat, has been found to significantly reduce productivity [3]. With global warming causing an increase in average temperatures, high heat during the grain-filling stage affects grain production in many areas [3]. Heat stress is a major contributor to yield reduction, impacting more than 36 million hectares in temperate regions (Reynolds et al. 2001). A substantial portion of wheat cultivated in South Asia is believed to be affected by heat stress [4]. According to Bala et al. [5], high temperatures significantly reduce grain yield, the number of grains per kernel, plant height, grain filling duration, peduncle length, peduncle weight, and 1,000-kernel weight. Heat stress during the grain-filling phase shortens the grain growth period, leading to inadequate grain filling and negatively affecting the overall yield of the wheat crop [6].

Most crops that have been improved through conventional breeding techniques thrive under optimal conditions, which means that many agricultural varieties lack heat tolerance. As a result, it is essential to expedite biotechnological research aimed at creating heat-tolerant plants capable of adapting to local conditions, given their substantial contribution to agricultural production. The first step in breeding heat-tolerant varieties involves conducting studies that address various aspects, including reducing heat stress effects, molecular and biochemical characterization, and categorizing wheat varieties according to their heat tolerance levels. Bangladesh produces approximately one million tons of wheat each year. Despite its significance in global agriculture, certain areas in Bangladesh are vulnerable to heat stress due to the effects of climate change.

Wheat has a substantial genome size (16,000 Mb for bread wheat), making heat tolerance a complex quantitative trait regulated by multiple genes. To address this complexity, numerous studies have explored the molecular mechanisms underlying heat tolerance and molecular breeding approaches for heat resilience. Recently, several molecular markers and quantitative trait loci (QTLs) have been identified as being linked to genes involved in heat signaling mechanisms. Significant advancements have been achieved in the molecular identification of these key genes [7]. These advancements have enabled the development of heat-tolerant crops for the future, utilizing various molecular markers. Among these, DNA markers based on Polymerase Chain Reaction (PCR) are particularly significant. In wheat genetic characterization, several PCR-based molecular markers are employed, including amplified fragment length polymorphisms (AFLP) [8], sequence-tagged microsatellite site markers (STMSs) or simple sequence repeats (SSRs) [9], and chloroplast-specific microsatellite markers (CPSSR) [10]. For instance, Golabadi et al. [11] utilized microsatellite markers to identify QTLs associated with yield traits such as thousand grain weight and harvest index. Additionally, Ramya et al. [12] conducted physiological and genetic studies on 24 modern wheat genotypes to assess their drought and heat tolerance for breeding purposes. Based on the findings of these studies, SSR markers have been demonstrated to be effective in determining heat tolerance in wheat. High-yielding wheat genotypes that can withstand heat stress are identified by calculating the Heat Susceptibility Index (HSI) after evaluating various agronomic traits in field conditions [13,14,15,16]. The characterization of wheat genotypes for their tolerance to high temperatures has revealed those with superior relative performance in yield components, overall grain yield, and HSI [17,18]. Several studies have also documented the application of HSI and performance assessments under late sowing conditions with heat stress [14,19], (Yang et al. 2010; Barakat et al. 2011), [15,16]. To conduct genetic analyses through QTL studies, it is essential to identify genotypes that exhibit contrasting traits. This study aims to characterize bread wheat genotypes for their heat tolerance and adaptability to various local conditions in Bangladesh.

2. MATERIALS AND METHODS

2.1 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 [20]. 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.

2.2 Extraction of DNA and SSR Analysis

Genomic DNA was extracted from fresh leaves of 15 wheat genotypes. Total genomic deoxyribonucleic acid (DNA) from each genotype was isolated from young seedling leaves using a modified version of the cetyl trimethyl ammonium bromide (CTAB) extraction method. 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).

The fresh leaves of each genotype were ground using a mortar and pestle, then transferred to a 2 ml centrifuge tube. Chloroform (400 µl) was added and gently mixed by inverting the tube, followed by heating in a water bath at 65ºC for 1 hour. The sample was then centrifuged at 12,000 rpm for 10 minutes at 4ºC. The supernatant was carefully transferred to a new 1.5 ml tube, and an equal volume of isopropyl alcohol (isopropanol) was added, followed by gentle mixing through inversion (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 utilized to assess the concentration and quality of the extracted DNA prior to Polymerase Chain Reaction (PCR) amplification. Thirteen primer pairs were employed for the SSR analysis, with PCR conditions set according to the methodology outlined by Roder et al. (1998).

Each PCR reaction was conducted in a total volume of approximately 10 µl, which included nuclease-free water, master mix, and the specific primer pairs in accordance with their profiles. The amplification of wheat genomic DNA involved an initial incubation of the samples for 5 minutes at 94°C, followed by 45 cycles consisting of denaturation at 94°C for 60 seconds, primer annealing at 58-60°C for 60 seconds, and extension at 72°C for 60 seconds. A final extension step was conducted for 10 minutes at 72°C.

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		2020	-	-
WMRI Gom 3		2020	-	-
BAW 1290		-	-	-
BAW 1147		-	-	-
Nadi 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

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

2.3 Statistical analysis

Each band was treated as a distinct locus, and all the scorable loci were utilized to create a bivariate 1-0 data matrix. Genetic distances (GD) among the genotypes were calculated using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) based on shared alleles. To assess genetic diversity, a dendrogram and the Polymorphism Information Content (PIC) values were generated using PowerMarker software.

2.4 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:

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

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

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

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

3. RESULTS AND DISCUSSION

In this study, a total of 13 SSR primers were utilized (refer to Table 2, Fig. 1). The allele counts and sizes for each primer are detailed in Table 3. The number of alleles identified among the bread wheat genotypes varied from 2 to 8. The most polymorphic microsatellite marker was Gwm293, which displayed 8 alleles, closely followed by Xgwm356, which revealed 7 alleles (Table 3). Overall, 51 polymorphic alleles were identified from the 15 bread wheat genotypes screened using these 13 SSR markers, resulting in an average of 3.92 alleles per locus. The primer Xcfd43 exhibited the lowest number of alleles, with only 2 detected.

The polymorphism information content (PIC) values for the microsatellite markers analyzed ranged from 0.325 (Xwmc407) to 0.827 (Xgwm356), with a mean PIC value of 0.567, which aligns closely with findings reported by Zheng et al. (2009). Among the 13 SSR markers assessed in this research, the Xgwm356 primer yielded the highest PIC value of 0.827, followed by Gwm293, which had a PIC value of 0.818. The lowest PIC value was observed for the Xwmc407 primer, which had a PIC value of 0.325. 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 employed in this research reveal a low level of heterozygosity, with an average expected heterozygosity (He) value of 0.372 among the wheat genotypes examined, indicating limited genetic variation. Out of the primers used, 13 loci were deemed informative, as they exhibited a PIC value exceeding 0.5. The PIC value serves as an indicator of genetic variability within a plant. Specifically, loci with a PIC value above 0.5 are classified as having high diversity, whereas those with a PIC below 0.25 are categorized as exhibiting low diversity [21,22,23]. In this study, the average PIC value for the SSR markers was 0.567, with a range from 0.325 to 0.827.

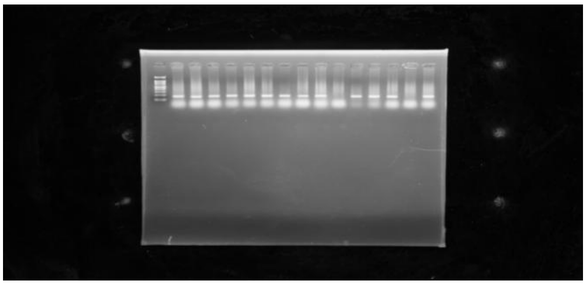
Thus, the majority of primers used in this study proved to be highly informative. The results from the SSR analysis indicate that these markers have strong potential for use in marker-assisted selection for terminal heat stress tolerance through molecular plant breeding. According to Hao et al. [24], both the number of alleles at each locus and the calculated polymorphism information content (PIC) values should be jointly evaluated to provide an accurate assessment of genetic diversity within genotype collections. Since PIC values are positively correlated with allele numbers across all genotypes, this study found a significant positive correlation between PIC values and the allele range of the microsatellites evaluated.

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

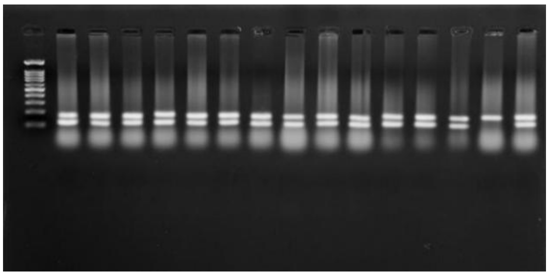
Marker	Allele No	Allele size and range	Difference (bp)	Major.Allele Frquency	Gene Diversity	Heterozygosity (He)	PIC
gwm291	3	150-160	10	0.4615	0.639	0.000	0.566
Gwm325	3	150-160	10	0.3750	0.656	0.000	0.582
Xgwm294	4	50-120	70	0.5000	0.645	1.000	0.587
Gwm268	3	180-285	105	0.6667	0.475	0.111	0.404
Xwmc407	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**

Table 4. Genetic Distance of 15 wheat genotypes based on 13 SSR markers

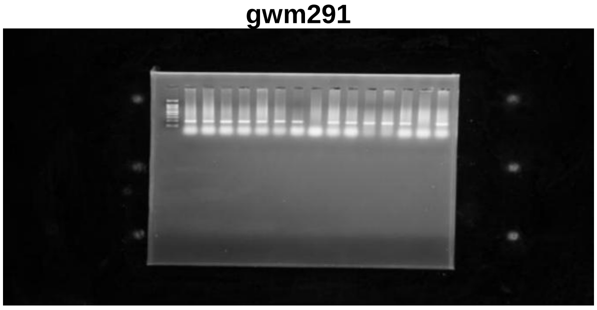
	BARI Gom 25	BARI Gom 26	BARI Gom 27	BARI Gom 28	BARI Gom 29	BARI Gom 30	BARI Gom 31	BARI Gom 32	BARI Gom 33	BAW 1147	BAW 1290	Nadi 2	WMRI Gom 1	WMRI Gom 2	WMRI Gom 3
BARI Gom 25	0.000														
BARI Gom 26	0.150	0.000													
BARI Gom 27	0.545	0.500	0.000												
BARI Gom 28	0.583	0.500	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



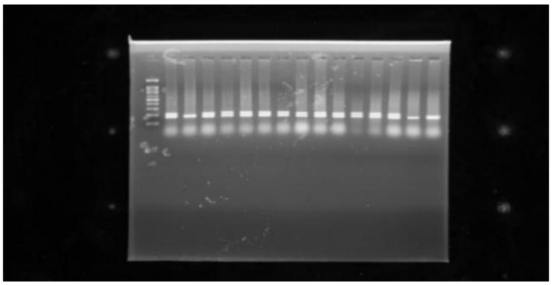
gwm291



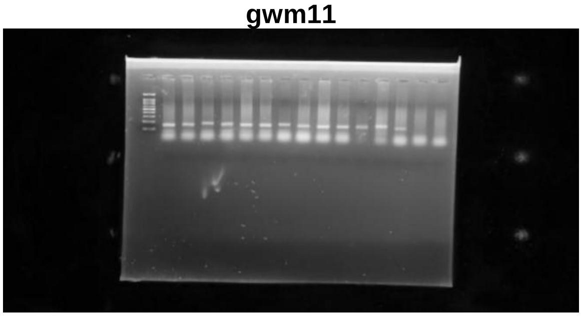
Xcfa2129



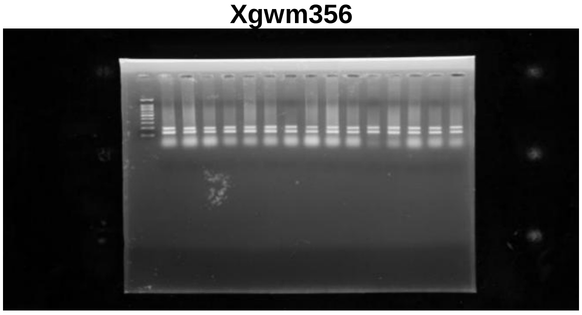
gwm11



Xgwm356



Gwm484



Gwm293

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

The highest genetic diversity (0.847) was observed at locus Xgwm356, while the lowest (0.439) was observed at locus gwm11, with an average diversity value of 0.629 (Table 3). Markers detecting fewer alleles exhibited lower gene diversity, whereas markers detecting a higher number of alleles demonstrated greater gene diversity. This finding aligns with previous research by Herrera et al. [25], who also reported a significant correlation between genetic diversity at SSR loci and the number of alleles detected by microsatellite markers.

Pairwise comparisons of shared alleles and genetic distance (D) between varieties, computed from the combined data for 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). A higher genetic distance between varieties suggests greater genetic diversity, whereas a lower genetic distance indicates closer genetic similarity. Essentially, this value reflects the genetic dissimilarity between varieties, with higher values representing more dissimilar pairs and lower values representing more similar pairs. The lowest genetic distance (0.000) was observed between the Nadi 2 and BAW1147 variety pair, indicating that these two varieties are genetically identical.

A dendrogram was constructed based on the genetic distance calculated from 51 alleles across 15 wheat varieties, enabling the clear differentiation of all 15 cultivars. The UPGMA cluster tree analysis grouped the 15 wheat varieties into four main clusters (Fig. 2). The dendrogram divided the varieties into two broad groups, A and B, which emerged at a distance coefficient of 0.05. Group A, consisting of seven genotypes, was further divided into two clusters, while Group B, consisting of eight genotypes, was similarly subdivided into two clusters. The cluster-wise mean values for heat susceptibility index (HSI), thousand grain weight (TGW), and grain yield, both in optimal and late-sown conditions, as well as the percentage change in TGW and grain yield between late-sown and timely-sown conditions, are presented in Tables 5 and 6. These two major groups differed in three key parameters related to field-level heat tolerance: HSI, TGW, and grain yield under stress conditions, and the percent reduction in TGW and grain yield under stress compared to normal conditions, as indicated in Tables 5 and 6.

The Heat Susceptibility Index (HSI) was measured for TGW and grain yield to identify heat-tolerant and heat-susceptible genotypes. HSI estimates for all genotypes revealed the presence of both resistant and susceptible varieties. The HSI for TGW ranged from 0.772 to 1.218, while for grain yield, it ranged from 0.742 to 1.253. These values were used to identify heat-tolerant genotypes. A lower HSI value (below 1) indicates higher stress tolerance [26]. Based on the HSI values, genotypes were classified as highly heat tolerant (HSI below 0.50), moderately heat tolerant (HSI 0.50-1.00), and heat susceptible (HSI above 1.00) [27], (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).Thous and 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.

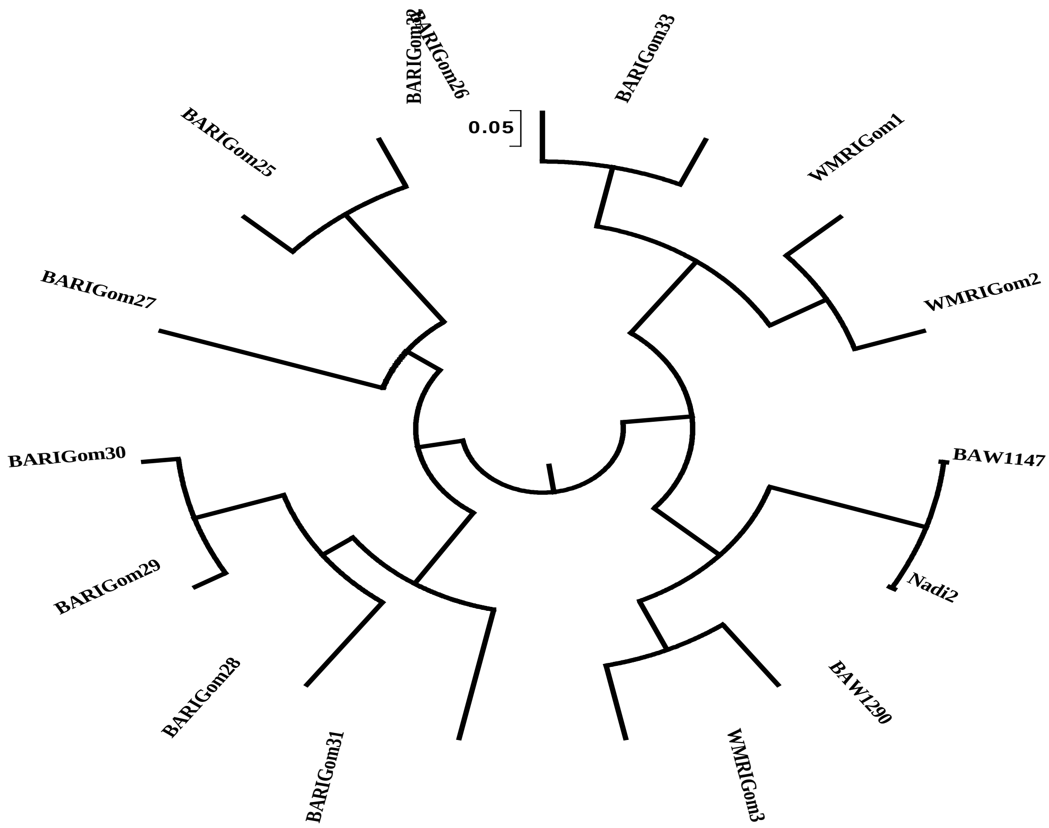


Fig. 2. Dendrogram generated through UPGMA analysis showing genetic relationship among the 15 wheat genotypes. Names of the genotypes are given on the ends of branches

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				

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 (HIS above 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 (HIS above 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., [28], Pinto et al. [19] and Sadat et al. [20] who used SSR markers for assessing the genetic diversity for heat stress tolerance in wheat.

A review of the four clusters revealed that Cluster IV exhibited the highest mean HSI value and the greatest reduction in TGW under late-sown conditions compared to timely sown conditions. Similarly, Cluster III had the highest mean HSI value and the largest decrease in grain yield under late-sown conditions. Despite this, BARI Gom 25 and BARI Gom 30 demonstrated genetic potential for higher yields, as evidenced by their superior performance under stress compared to other genotypes in Group A. The molecular classification of BAW 1147 as a heat-sensitive genotype is supported by its high HSI value for grain yield. Genotypes BARI Gom 25, BARI Gom 28, BARI Gom 29, BARI Gom 30, and BARI Gom 31 from Group A have shown their suitability for late-sown conditions. Consequently, the morphological data for most of these genotypes aligned with the molecular findings.

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 observed dissimilarities may be attributed to the regional nature of heat stress. In some regions, the stress affects plants for only a few hours, while in others, it persists from the reproductive stage until the wheat matures. Additionally, heat stress is a complex trait that interacts with another intricate trait, yield, leading to genotype × environment interactions that significantly influence the expression of yield traits. As the genotypes were assessed under field conditions, the variations in weather were evident, aligning with findings reported by Pandey et al. [29]. Developing heat-tolerant wheat varieties has become a key focus in agricultural research since temperatures exceeding the optimal range (21.3±1.27˚C) during the reproductive phase, particularly during grain filling, can severely impact wheat yields. Thus, there is an urgent necessity to identify or develop genotypes that can either withstand terminal heat stress or mature early without considerable yield reductions [30-32].

4. CONCLUSION

The molecular and genetic approaches utilized in this study, which identified DNA polymorphisms associated with thermotolerance, will not only enhance marker-assisted breeding for heat tolerance but also facilitate the cloning and characterization of essential genetic factors that could be beneficial for engineering plants with improved heat tolerance. In summary, the SSR markers applied in this investigation demonstrated their efficacy in classifying wheat genotypes as either susceptible or tolerant to terminal heat stress, with only a few exceptions.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies have been used during writing or editing of this manuscript.

ACKNOWLEDGMENT

The authors wish to express their gratitude to the Bangladesh Wheat and Maize Research Institute (BWMRI), Bangladesh Agricultural Research Institute (BARI) for providing research facilities and Genetic materials, International Maize and Wheat Improvement Centre (CIMMYT) for providing few of the genetic materials and Krishi Gobeshona Foundation (KGF) for providing Research Grant (BR5, C/17) for the successful completion of these researches

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mickelbart MV, Hasegawa PM, Bailey-Serres J. Geneticmechanisms of abiotic stress tolerance that translate to crop yield stability. Nat Rev Genet. 2015;16(4):237–251.
2. Bita C, Gerats T. Plant tolerance to high temperature in achanging environment: scientific fundamentals and production ofheat stress-tolerant crops. Front Plant Sci. 2013;4:273
3. Hays D, Mason E, Do JH, Menz M, Reynolds M. Expression quantitative trait loci mapping heat tolerance during reproductive development in wheat (*Triticum aestivum*). In Wheat production in stressed environments Springer Netherlands. 2007;373-382.
4. Joshi AK, Chand R, Arun B, Singh RP, Ortiz-Ferrara G. Breeding crops for reduced-tillage management in the intensive, rice–wheat systems of South Asia. Euphytica. 2007;153:135–151.
5. Bala S, Asthir B, Bains NS. Effect of terminal heat stress on yield and yield attributes of wheat. Indian Journal of Applied Research. 2014;4(6):1-2.
6. Rane J, Pannu RK, Sohu VS, Saini RS, Mishra B et al. Performance of yield and stability of advanced wheat genotypes under heat stressed environments of Indo-Gangetic Plains. Crop Science. 2007;47:1561–1573.
7. Yıldırım A, Ates¸ So¨nmezog˘lu O¨, Sayaslan A, Koyuncu M, Gu¨lec T, Kandemir N. Marker-assisted breeding of a durum wheatcultivar for gliadin and LMW-glutenin proteins affecting pasta quality. Turk J Agric For. 2013;37:527–533.
8. Barrett BA, Kidwell KK. AFLP-based genetic diversity assessment among wheat cultivars from The Pacific Northwest.Crop Sci. 1998;38:1261–1271.
9. Prasad M, Varshney RK, Roy JK, Balyan HS, Gupta PK. Theuse of microsatellites for detecting DNA polymorphism, genotype identification and genetic diversity in wheat. Theor Appl Genet. 2000;100:584–592.
10. Tomar RS, Deshmukh RK, Naik KB, Tomar SMS. Development of chloroplast-specific microsatellite markers for molecularcharacterization of alloplasmic lines and phylogenetic analysis in wheat. Plant Breed. 2013;133(1):12–18.
11. Golabadi M, Arzani A, Mirmohammadi Maibody SAM, Sayed Tabatabaei BE, Mohammadi SA. Identification of microsatellite markers linked with yield components under drought stress at terminal growth stages in durum wheat. Euphytica. 2011;177:207–221.
12. Ramya P, Jain N, Singh PK, Singh GP, Prabhu KV. Population structure, molecular and physiological characterisation of elite wheat varieties used as parents in drought and heat stress breeding in India. Indian J Genet Plant Breed. 2015;75(2):250–252.
13. Kirigwi FM, van Ginkel M, Brown-Guedira G, Gill BS, Paulsen GM, Fritz AK. Markers associated with a QTL for grain yield in wheat under drought. Mol Breeding. 2007; 20:401-413.
14. Mohammadi V, Zali AA, Bihamta MR. Mapping QTL for heat tolerance in wheat. Journal of Agricultural Science and Technology. 2008;10:261-267.
15. Mason RE, Mondal S, Beecher FW, Pacheco A, Jampala B, Ibrahim AMH, Hays DB. QTL associated with heat susceptibility index in wheat (*Triticum aestivum* L.) under short-term reproductive stage heat stress. Euphytica. 2010; 174:423-436.
16. Mason ER, Mondal S, Beecher WF, Hays DB. Genetic loci linking improved heat tolerance in wheat (*Triticum aestivum* L.) to lower leaf and spike temperature under controlled conditions. Euphytica. 2011; 180:181-194.
17. Rahman MA, Chikushi J, Yoshida S, Karim AJMS. Growth and yield components of wheat genotypes exposed to high temperature stress under control environment. Bangladesh J. Agril. Res. 2009;34(3):361-372.
18. Khan AA, Shamsuddin AKM, Barma NCD, Alam MK, Alam MA. Screening for heat tolerance in spring wheat (*Triticum aestivum* L.). Tropical Agricultural Research and Extension. 2014;17(1):26-37.
19. Pinto S, Chapman SC, McIntyre CL, Shorter R, Reynolds M. For canopy temperature response related to yield in

both heat and drought environments. Theor. Appl. Genet. 2010;121(6):1001-1021.
20. Sadat S, Saeid KA, Bihamta MR, Torabi S, Salekdeh SGH, Ayeneh GAL. Marker assisted selection for heat tolerance in bread wheat. World App. Sci. J. 2013;21(8):1181–1189.
21. Botstein D, White R, Skolnick M, Davis R. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet. 1980;32:314–331.
22. Nagy S, Poczai P, Cerna´k I, Gorji AM, Heged}us G, Taller J. PICcalc: An online program to calculate polymorphic information content for molecular genetic studies. Biochem Genet. 2012;50(9–10):670–672.
23. Ramadugu C, Keremane ML, Hu X, Karp D, Federici CT, Kahn T, Lee RF. Genetic analysis of citron (*Citrus medica* L.) using simple sequence repeats and single nucleotide polymorphisms. Sci Hortic. 2015;195:124–137.
24. Hao CY, Zhang XY, Wang LF, Dong YS, Shang XW, Jia JZ. Genetic diversity and core collection evaluations in common wheat germplasm from the northwestern spring wheat region in China. Mol Breed. 2006;17(1):69–77.
25. Herrera TG, Duque DP, Almeida IP, Nunez GT Tohme. Assement on genetic diverdity in Venezuelan rice ciltivars using simple sequence repeats markers. Electron. J. Biotechnol. 2008;11(5):215-226.
26. Fischer RA, Maurer O. Crop temperature modification and yield potential in a dwarfing spring wheat. Crop Sci. 1978; 16:855-859.
27. Khanna-Chopra R, Viswanathan C. Evaluation of heat stress tolerance in irrigated environment of T. aestivum and related species. I. Stability in yield and yield components. Euphytica. 1999;106: 169-180.
28. Ali RA, Kelestanie A, Asadi A, Mirfakhraei SR, Abasi AR. Genetic diversity in twenty bread wheat cultivars using microsatellite markers. Int. J. Agro. and Plant Production. 2013;4(8):1920–1927.
29. Pandey GC, Rane J, Sareen S, Siwach P, Singh NK, Tiwari R. Molecular investigations on grain filling rate under terminal heat stress in bread wheat (*Triticum aestivum* L.). African j. of Biot. 2013;12(28):4439–4445.
30. Ates So¨nmezog˘lu O¨, Balkan AS. Molecular and biochemical analysis of durum wheat genotypes to examine carotenoid pigment content and lipoxygenase enzyme activity. Cereal Res Commun. 2014;42(2):218–228.
31. Paliwal R, Roder MS, Kumar U, Srivastava JP, Joshi JP. QTL mapping of terminal heat tolerance in hexaploid wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics. 2012;125:561-575.
32. Ro¨der MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW. A microsatellite map of wheat. Genetics. 1998;149(4):2007–2023.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of the publisher and/or the editor(s). This publisher and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

© Copyright (2024): Author(s). The licensee is the journal publisher. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/125288