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# Molecular characterization and identification of unique alleles for thermo-tolerance in wheat varieties

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## Abstract

*Genetic diversity of wheat cultivars is very important in reducing genetic vulnerability especially during high temperature stress. This study was conducted to estimate genetic diversity among wheat varieties and to identify the simple sequence repeats (SSR) markers linked to the high temperature stress tolerance in wheat. For this, wheat varieties already identified as thermo-tolerant and thermos-sensitive based on morpho-physiological parameters were characterized using SSR markers. Out of a total of 44 SSR markers used to study genetic diversity among 14 wheat varieties, 36 markers showed clear amplification pattern. The polymorphism percentage ranged from 33.3 to 100%, with an average value of 76.28%. The dendrogram prepared using NTSYSpc formed two major clusters at a similarity coefficient value of 0.68. At a similarity coefficient value of 0.751, all thermo-tolerant varieties clearly distinguished from thermo-sensitive ones. The microsatellite marker, Xgwm 46 amplified a 200 bp allele which was present in all the thermo-tolerant varieties but absent in all thermo-sensitive varieties. The identified SSR marker, Xgwm 46 was found to be thermo-tolerance specific and could be of potential use in wheat improvement programs through marker assisted selection.*

**Keywords:** Genetic diversity, SSR markers, Thermo-tolerance, Wheat

## 1. Introduction

Wheat (*Triticum aestivum* L.) is the staple food to one third of the global population which makes it most important among field crops. It is cultivated under diverse environmental conditions varying from cool rain-fed to hot dry-land areas. In many areas of the world, high temperature stress is the major limiting factor for wheat production. The crop experiences varying degree of heat stress at different phenological stages, but reproductive phase is more sensitive to higher temperature than vegetative stage due to its direct effect on grain number and dry weight (B. WOLLENWEBER & al. [1]). The optimum temperature required for wheat anthesis and grain filling is from 12 to 22°C (H. TEWOLDE & al. [2]). Increase in temperature beyond optimum reduces the duration between anthesis and the physiological maturity, which is further associated with a reduction in grain weight (L. SHPILER & al. [3], B. LIU & al. [4]). Combined yield loss caused by heat stress is around 40 million tones for wheat, corn and barley per year, where wheat alone accounts for almost half of the yield loss (D.B. LOBELL & al. [5]). Global wheat production is estimated to fall by 6% for each degree Celsius of further increase in global temperature (S. ASSENG & al. [6]).

Availability of diverse genetic resources and the ability to maintain yield across different environments makes a crop improvement program really effective. It becomes more and more significant under the harsh and fluctuating climatic conditions leading to high genotype and environment interactions. Substantial increase in genetic diversity has been reported in wheat after the 1990s, may be due to utilization of diverse parents and wild relatives in breeding programs and/or due to availability of better selection methods to estimate genetic diversity including molecular markers. The genetic diversity of hexaploid wheat is maintained in India, Austria, Albania and Nepal since Genebank activities started in last century (A. BORNER & al. [7]). Polyploidy nature is also partially responsible for maintaining genetic diversity of wheat (M.K. KHAN & al. [8]). The utilization of this available genetic diversity depends upon identification of trait specific germplasm and its further molecular characterization to find out the linked markers for use in crop improvement program.

Estimation of genetic diversity by morphological and biochemical traits is laborious, time consuming and influenced by the environmental conditions. Molecular markers have been proved to be powerful tool to study genetic diversity of wheat as the marker can directly characterized the genome of organism and they are not influenced by the environment (X. SUN & al. [9]). Among PCR based molecular markers, SSRs (Simple Sequence Repeats) particularly have been applied extensively to estimate genetic diversity among wheat cultivars because of their genome specificity, co-dominant, multi-allelic highly reproducible nature, relative abundance and uniformly distribution in genome (P.K. GUPTA & al. [10]). They have been used for various applications including characterization of wheat varieties (M.M. MANIFESTO & al. [11]), identification of marker linked to particular trait (M.A. BARAKAT & al. [12]), marker assisted selection (T. MIEDANER & al. [13]) and identification of quantitative trait loci (J. YANG & al. [14], S.K. TALUKDER & al. [15]). Hence, this study was carried out to find out genetic diversity among thermo-tolerant and thermos-sensitive wheat varieties using SSR markers and to identify unique alleles associated with thermo-tolerance which can be used for developing thermo-tolerant wheat varieties using marker assisted selection.

## 2. Materials and Methods

### Plant material and DNA extraction

Fourteen wheat varieties were undertaken as the experimental material (Table 1). The selection of these wheat varieties (7 thermo-tolerant and 7 thermo-sensitive) was based on the phenological and physiological studies related to high temperature stress tolerance. The seeds of these 14 wheat varieties were procured from the Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar, India and plants were raised in a net-house. Genomic DNA was isolated from young leaves of 2 to 3 week-old plants using CTAB (Cetyl Trimethyl Ammonium Bromide) method (M.A. SAGHAI-MAROOF & al. [16]). DNA was checked for its quality and quantity by measuring absorbance at 260 and 280 nm using spectrophotometer.

**Table 1.** A brief description of 14 wheat varieties used in this study

| Varieties | Type of genotype | Code |
|---|---|---|
| UP 2338 | Thermo-sensitive | S1 |
| DBW 17 | Thermo-sensitive | S2 |
| PBW 550 | Thermo-sensitive | S3 |
| WH 283 | Thermo-sensitive | S4 |
| HD 2687 | Thermo-sensitive | S5 |
| WH 711 | Thermo-sensitive | S6 |
| WH 542 | Thermo-sensitive | S7 |
| WH 1022 | Thermo-tolerant | T1 |
| GW173 | Thermo-tolerant | T2 |

| | | |
|---|---|---|
| RAJ 3765 | Thermo-tolerant | T3 |
| WH 730 | Thermo-tolerant | T4 |
| WH 1021 | Thermo-tolerant | T5 |
| NIAW 34 | Thermo-tolerant | T6 |
| WH 1076 | Thermo-tolerant | T7 |

**PCR amplification using SSR markers**

The polymerase chain reaction was carried out in a BIORAD$^{TM}$ Thermal Cycler using SSR markers listed in Table 2. Forty-four SSR markers (Xgwm, WMC and Xbarc series) were selected based on highly polymorphic nature of markers from earlier studies (M.S. RODER & al. [17], P.K. GUPTA & al. [18], D.J. SOMERS & al. [19]) used to analyze genetic diversity of 14 wheat varieties. The PCR reaction mixture (20µl) included: 50 ng template DNA, 1X PCR buffer, 200 µM of each dNTPs, 1.5 mM $MgCl_2$, 0.25 µM each of forward and reverse primers and 1.0 U Taq DNA polymerase. The PCR profile started with 94°C for 4 min; followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 55 to 65°C (depending on primer) for 1 min and extension at 72°C for 2 min. A final extension 72°C for 15 min was included. The PCR products were resolved by 3% agarose gel electrophoresis at 80 V for 2 to 3 h. The gel was stained with ethidium bromide and then observed on gel documentation system (Alpha Innotech Corporation). The PCR products which could not be resolved on agarose gel were resolved on 4% polyacrylamide gels and visualized via silver staining protocol (PROMEGA Inc. Madison, WI, USA) to achieve better resolution of the alleles.

**Data analysis**

All the wheat varieties were scored for the presence and absence of the alleles in the gel. The data were entered into a binary matrix as discrete variables, 1 for presence and 0 for absence of the allele. The 0/1 matrix was used to calculate the similarity matrices using 'Simqual' subprogram of numerical taxonomy and multivariate analysis system program (NTSYS-pc 2.0) software (F.J. ROHLF [20]). Based on the similarity matrix, dendrogram was constructed using unweighted pairgroup method with arithmetic average (UPGMA) sub-programme of NTSYS-pc 2.0.

## 3. Results and Discussion

**SSR marker analysis**

A total of 44 SSR markers were used to screen the 14 wheat varieties varying for thermo-tolerance. Most of primers used in this study revealed a high polymorphism. Out of 44 SSR markers used, only 36 markers produced clear amplification profiles. Using these 36 SSR markers, 91 alleles were amplified, out of which 73 were polymorphic. The number of alleles detected ranged from 1 (Xgwm 157, Xgwm 293, Xgwm 388 and Xgwm 458) to 6 (Xgwm 132) with a mean value of 2.53 alleles per locus. This revealed significant allelic diversity among various microsatellite loci. (I. SCHUSTER & al. [21]) reported similar results in a study on 36 wheat genotypes using 23 SSR markers. The total number of amplified allele varied from 2 to 5 with an average of 3.2 alleles per locus, which is close to results of present investigation. A. AKRAM & al. [22] scored 3 to 9 alleles per locus with an average value of 6.02, whereas, M. NEFZAOUI & al. [23] scored 2.6 alleles per locus. Y. TEKLU & al. [24] investigated genetic diversity in 73 accessions of emmer wheat (*Triticum dicoccon* Schrank) from 11 geographical regions using a set of 29 SSR markers and reported 12.31 alleles per locus. This difference in number of alleles per locus may be due to the fact that genotypes were selected from different geographical regions and different SSR markers were evaluated in these studies. Some markers like WMC 170 (Figure 1), Xgwm 165, Xgwm 190, Xgwm 400, Xgwm 428, and Xgwm 539 did not show polymorphism on agarose gel, which were resolved on polyacrylamide gel electrophoresis. The SSR markers showed a high level of polymorphism ranging from 33.3 to 100% with an average polymorphism of 76.28%. The size of PCR amplified products ranged between 95 bp and 1120 bp. The polymorphism generated among various wheat varieties using these SSR markers is presented in Table 2.

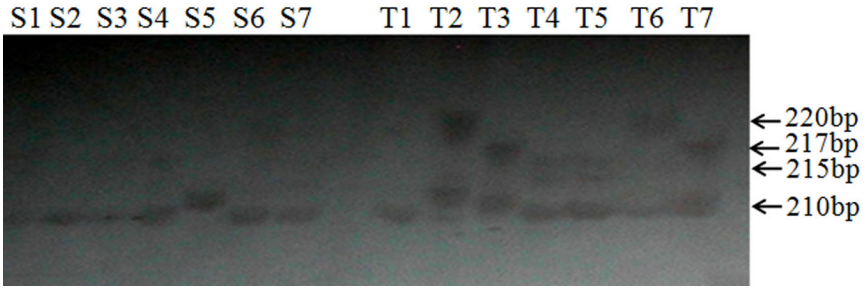


**Figure 1.** SSR banding profile of 14 wheat varieties with WMC 170 marker on polyacrylamide gel (S1-S7: Thermo-sensitive wheat varieties, T1-T7: Thermo-tolerant wheat varieties)

**Table 2.** DNA amplification profile and polymorphism generated in 14 wheat varieties using 36 SSR markers

| SSR Markers | Chromosome location | Size of of alleles | Total no. of alleles | Number of polymorphic alleles | Percentage polymorphism |
|---|---|---|---|---|---|
| Xgwm 18 | 1B | 100-215 | 2 | 2 | 100 |
| Xgwm 46 | 7B | 150-200 | 2 | 2 | 100 |
| Xgwm 95 | 2A | 110-140 | 2 | 2 | 100 |
| Xgwm 102 | 2D | 140-170 | 3 | 3 | 100 |
| Xgwm 111 | 7D | 120-310 | 4 | 4 | 100 |
| Xgwm 131 | 1B/3B | 110-350 | 3 | 3 | 100 |
| Xgwm 132 | 6B | 125-1120 | 6 | 5 | 83.3 |
| Xgwm 157 | 2D | 105 | 1 | 0 | 0 |
| Xgwm 165 | 4A/4B | 125-155 | 3 | 2 | 66.6 |
| Xgwm 186 | 5A | 110-185 | 2 | 2 | 100 |
| Xgwm 190 | 5D | 105-125 | 2 | 2 | 100 |
| Xgwm 261 | 2D | 165-195 | 2 | 2 | 100 |
| Xgwm 293 | 5A | 195 | 1 | 0 | 0 |
| Xgwm 311 | 2A/2D | 115-190 | 2 | 2 | 100 |
| Xgwm 332 | 7A | 95-185 | 3 | 1 | 33.3 |
| Xgwm 356 | 2A | 190-225 | 2 | 1 | 50 |
| Xgwm 388 | 2B | 150-190 | 2 | 0 | 0 |
| Xgwm 389 | 3B | 100-155 | 2 | 2 | 100 |
| Xgwm 400 | 7B | 105-135 | 2 | 2 | 100 |
| Xgwm 408 | 5B | 160-215 | 3 | 3 | 100 |
| Xgwm 428 | 7D | 140-750 | 3 | 2 | 66.6 |
| Xgwm 455 | 2D | 125-310 | 5 | 4 | 80 |
| Xgwm 458 | 1D | 110 | 1 | 0 | 0 |
| Xgwm 526 | 2B | 120-170 | 2 | 1 | 50 |
| Xgwm 539 | 2D | 140-165 | 3 | 2 | 66.6 |
| Xgwm 601 | 4A | 120-920 | 2 | 2 | 100 |
| Xgwm 610 | 4A | 185-210 | 2 | 1 | 50 |
| Xgwm 617 | 5A/6A | 105-195 | 2 | 2 | 100 |
| WMC 48 | 4A | 105-205 | 3 | 2 | 66.6 |
| WMC 89 | 4B | 100-265 | 3 | 2 | 66.6 |
| WMC 120 | 2B | 140-165 | 2 | 2 | 100 |
| WMC 169 | 3A | 115-200 | 2 | 2 | 100 |
| WMC 170 | 2A/2D | 210-220 | 4 | 4 | 100 |
| WMC 215 | 5D | 185-220 | 2 | 2 | 100 |
| WMC 382 | 2B | 120-210 | 3 | 3 | 100 |
| Xbarc188 | 6B | 160-215 | 3 | 2 | 66.6 |
| **Average** | | | **2.53** | **2.03** | **76.28** |

**Genetic diversity analysis**

Genetic diversity is very crucial for adaptability studies and section of wheat genotypes for developing mapping population under heat stress conditions. Parents for crossing should be selected based on their genetic distance, to maximize the potential for genetic gain in the mapping population (N.H. NIELSEN & al. [25]). In the present study, 7 thermo-tolerant and 7 thermo-sensitive wheat varieties were used for hierarchical cluster analysis and 14 wheat varieties were grouped into two major clusters at a similarity coefficient value of 0.68 (Figure 2). Cluster-I was occupied by the thermo-sensitive wheat varieties namely, UP 2338 (S1), WH 283 (S4), DBW 17 (S2) and WH 711 (S6) while cluster-II grouped three thermo-sensitive *viz.* PBW 550 (S3), WH 542 (S7) and HD 2687 (S5); and all the seven thermo-tolerant varieties. Cluster-II further formed two different groups at a similarity coefficient value of 0.751 which separated all thermo-sensitive varieties from the thermo-tolerant ones. Presence of most of the thermo-tolerant varieties in close proximity in the dendrogram separated from thermo-sensitive varieties showed that they are genetically distinct from thermo-sensitive varieties. The similarity matrices of different wheat varieties ranged from 0.60 to 0.90. Maximum similarity (90%) was observed between PBW 550 (S3) and WH 542 (S7) wheat varieties. Wheat varieties, UP 2338 (S1) and GW 173 (T2); WH 283 (S4) and RAJ 3765 (T3) were found to be genetically most diverse with similarity value of 0.60. A high level of genetic similarity was observed among the wheat varieties with an average similarity coefficient value of 0.72. Thermo-sensitive as well as thermo-tolerant varieties showed maximum similarity among themselves. Higher genetic similarity highlighted the narrow genetic base of the wheat germplasm. Similar observations were reported earlier by different workers using SSR markers (F.S. AKFIRAT & al. [26]). Hence, genetic diversity estimate can be used as a valuable tool for selecting diverse parental genotypes for developing mapping population which could be further used for identification of QTLs linked to thermo-tolerance.

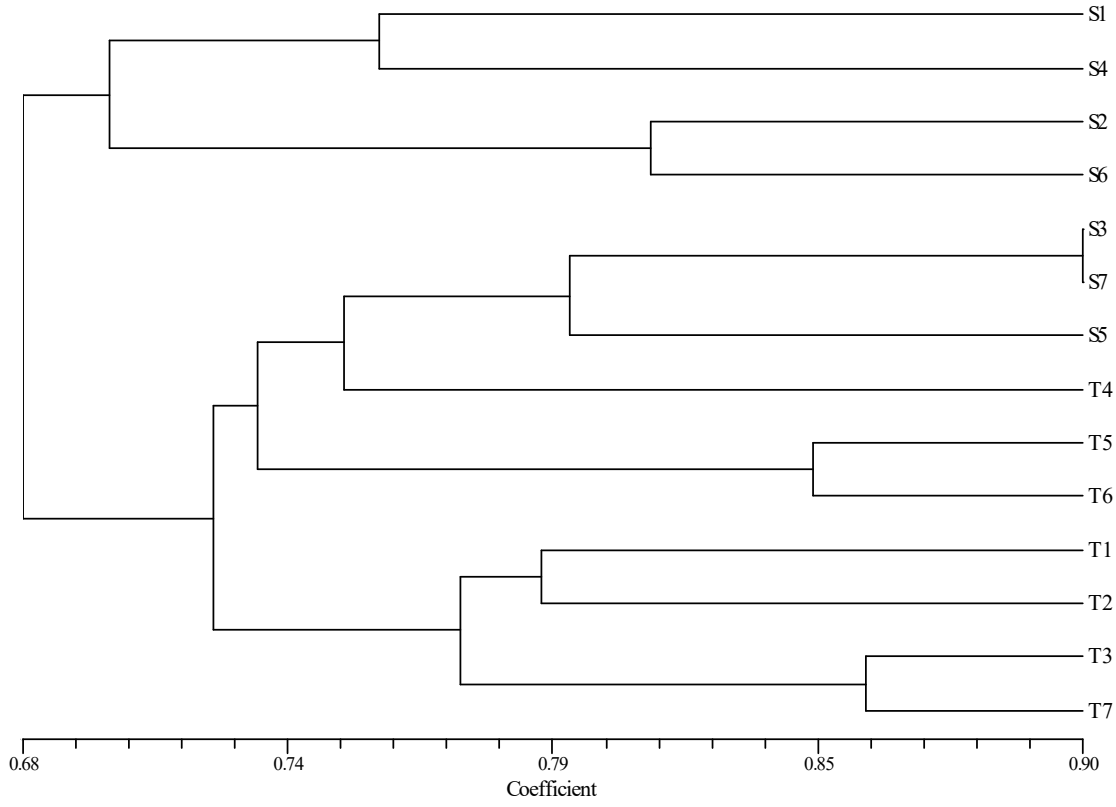


**Figure 2.** Dendrogram constructed with UPGMA clustering method among 14 wheat varieties using SSR markers

**Thermo-tolerance specific marker**

Among all the 36 SSR markers screened in 7 thermo-tolerant and 7 sensitive wheat varieties, a SSR marker Xgwm 46 on chromosome 7B produced a thermo-tolerance specific allele. Xgwm 46 amplified a 200 bp allele in all the seven thermo-tolerant varieties while no corresponding allele was amplified in any of the thermo-sensitive varieties (Figure 3). Many QTLs for thermo-tolerance in wheat have already been identified on chromosome 7B (V. MOHAMMADI & al. [27], R.S. PINTO & al. [28], M.A. BARAKAT & al. [12], R. PALIWAL & al. [29]). Hence, the above 200 bp amplified allele may be linked with already known or unknown QTL related to thermo-tolerance in wheat. It needs to be further validated and verify in mapping population. After validation, it could be utilized in the molecular breeding programs targeted to produce superior heat tolerant wheat varieties.

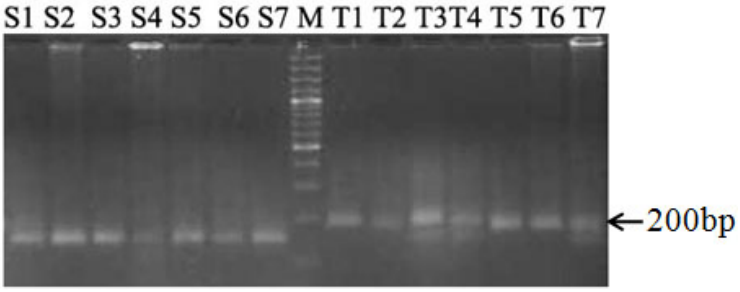


**Figure 3.** SSR banding profile of 14 wheat varieties with Xgwm 46 marker on agarose gel (S1-S7: Thermo-sensitive wheat varieties, M: 100 bp DNA ladder, T1-T7: Thermo-tolerant wheat varieties)

In conclusion, the present study revealed high genetic similarity among the wheat varieties under investigation which indicated their narrow genetic base. SSR markers used in the study were able to distinguish the thermo-tolerant wheat varieties from the thermo-sensitive ones. Further, specific alleles related to thermo-tolerance were obtained using Xgwm 46 marker. The thermo-tolerance specific marker obtained in the present study needs to be further validated and verified. After validation, it can be used to screen the wheat varieties for heat tolerance along with other already identified markers.

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