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Genetic analysis of yield and heat stress related traits in wheat (*Triticum aestivum* L. em. Thell) using microsatellite markers

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Abstract: Microsatellite markers were used for genetic analysis of terminal heat tolerance in F_2 (PBW373 × WH1081) population of wheat (*Triticum aestivum* L. em. Thell). Two parents were evaluated in field under normal sown and late sown conditions. For genotyping DNA from both parents PBW373 and WH1081 was amplified using 200 SSRs. Only 22 SSRs produced polymorphic bands, of size between 100 to 300 bp and an average of 1.45 alleles. The single marker analysis identified 19 markers indicating the putative QTLs for yield, its components and heat stress related physiological traits. The number of markers on these 16 linkage groups varied from one to four. On A genome 13 QTLs on B genome 5 QTLs and on D genome 9 QTLs were identified, respectively. The A, B and D genomes had 1360.3 cM, 272.4 cM and 919.5 cM of linkage coverage with average interval distances of 104.63 cM, 54.48 cM and 102.16 cM/Marker. A total of nine QTLs were resolved following composite interval mapping, one QTL was detected at a LOD score equal to threshold value of 2.5 while eight at LOD scores above the threshold value. All the nine QTLs were shown to be on definitive location on chromosome 3A (QDh.CCSHAU-3A, QDa.CCSHAU-3A and QPm.CCSHAU-3A), chromosome (QBm.CCSHAU-5A, QCtd.CCSHAU-5A and QCl.fl.CCSHAU-5A), chromosome6A (QPh.CCSHAU-6A) and chromosome3B (QTgw.CCSHAU and QMts.CCSHAU-3B). Use of these markers save times, resources and energy that are needed not only for raising large segregating populations for sveral generations, but also for estimating the parameters used for selection.

Keywords: Genotyping, QTL, MAS, Wheat

INTRODUCTION

Wheat is among the major three cereal crops, with over 600 million tones being produced annually. It is traditionally grown as a cool-season crop, but with the increased availability of more widely adapted germplasm, its production has expanded into warmer regions (Badaruddin *et al*., 1999). Continuous high-temperature stress for wheat has been defined as when the mean average temperature of the coolest month is greater than 17.5°C (Fischer and Byerlee, 1991), but there are many areas world wide where the coolest month temperature is higher. These areas are exposed to terminal heat stress, since there is rise in temperatures in grain filling period (Rane *et al*., 2007). Based on the special report, it is predicted that the annual daily maximum temperature is likely to increase by about 1-3^{0}C by mid-twenty-first century and by about 2-5^{0}C by the late twenty first century (IPCC, 2012). So, it is expected that future wheat will be exposed to higher heat stress. Wheat experiences heat stress to varying degrees at different phenological stages but heat stress during the reproductive phase is more harmful than during the vegetative phase as high temperature disturbs mobilization of resources to grain development leading to direct effect on grain number and grain weight (Wollenweber *et al*., 2003). Wheat production under late sown conditions in India is substantially low, due to heat stress during grain filling (Tewolde *et al.,* 2006). High temperatures shorten the grain filling period significantly in bread and durum wheat genotypes, because of significant interaction of each genotype with temperature (Dias and Lidon, 2009). With the development of methodologies for the analysis of plant gene structure and function, molecular markers have been utilized for identification of traits, to locate the gene(s) for a trait of interest on a plant chromosome and for the construction of genetic linkage maps. Direct selection under field conditions is generally difficult because uncontrollable environmental factors adversely affect the precision and repeatability of such traits. Assessment of heat tolerance at the molecular level is more meaningful than at phenotypic level as the later involves data on morphological traits which are environmental dependent. Available genetic diversity in wheat offers opportunity for the breeders to develop genotypes with wider adaptability having resistance to biotic and abiotic stresses by selection of recombinants of desired genes. The simple sequence repeats (SSR) markers can help breeders to

select genotypes carrying gene(s) of interest (Sadat *et al*., 2013), therefore, molecular maps based on these markers provide the breeders efficient strategies that may optimize time and resources and facilitate their manipulation in segregating plant breeding populations. These are powerful tools for many studies for genome characterization, detection of quantitative trait loci (QTL) for both abiotic and biotic stresses, evolutionary studies, and for marker assisted selection (MAS) (Peleg *et al*., 2008; Chu *et al*., 2010 and Sadat *et al*., 2013). In bread wheat, a variety of complex traits have been subjected to QTL analysis (Borner *et al.,* 2002; Wang *et al.,* 2009, 2010; Wu *et al.,* 2010; Rustgi *et al*., 2013) using SSR markers. Therefore, in present investigation genetic analysis of terminal heat tolerance was conducted to identify QTLs for stress related traits of wheat.

MATERIALS AND METHODS

Morphological characterization: A population of 152 F_2 plants from a cross of PBW373 × WH1081 was evaluated along with their parental genotypes and five check varieties (Raj3765, DBW17, WH730, WH711and PBW343) for phenological traits; days to heading, days to anthesis, days to physiological maturity, grain filling duration, plant height, number of productive tillers/plant, number of grains/spike, 1000 grain weight, grain yield/plant, biomass/plant, harvest index and physiological traits- canopy temperature depression, membrane thermostability and chlorophyll fluorescence. Parents and check varieties were sown in 3 meter paired rows in three replication in normal conditions, (E_1) on 29th November, 2011 and heat stress conditions, (E_2) on 3rd January, 2012 in randomized block design at wheat research area, Department of Genetics and Plant Breeding, CCS HAU, Hisar. F_2 population was grown in 10 rows each with two meter row length under heat stress environment (E_2). Row to row and plant to plant distance was kept at 25cm and 10cm, respectively, so as to raise the plants under space planting conditions. 60 kg N : 40 kg P_2O_5 and 40 kg K_2O per ha were applied at the time of sowing while 60 kg N per ha was top-dressed 21 days after sowing coinciding with crown root initiation. The observations were recorded on five randomly selected plants from each replication of parental genotypes and checks in both the environments, E_1 and E_2 (normal and heat stress conditions), single plant data was recorded on 152 F_2 plants of each of the cross PBW373 × WH1081 under stress conditions only.

Molecular characterization using SSR markers: Genomic DNA was isolated from each of parental genotypes, check varieties and F_2 plants using CTAB method of Saghai-Maroof *et al*. (1984). Agarose gel (0.8%) electrophoresis was used to check quality and quantity of genomic DNA by running DNA samples along with standard marker.

A total of 200 simple sequence repeats (SSR) markers widely distributed on different wheat chromosomes were used in this study. PCR amplification of genomic DNA from parents, check varieties and F_2 population was carried out in PCR machine 'Bench top lab systems-BT-B960' using following conditions: Initial Denaturation- 95°C for 5 min, denaturation-94°C for 1 min, Annealing-50°C to 65°C for 1min, Extension-72° C for 1 min, 35 cycles and Final Extension-72°C for 10 min. PCR amplified DNA products were resolved by submerged horizontal electrophoresis in 2.5% (w/v) agarose gel. For better resolution amplification products were also resolved on 6% polyacrylamide gels using Amersham Biosciences system as described by Chen *et al.* (1997).

SSR amplification profiles were scored visually, with A and B codes for presence of specific band in tolerant and sensitive parent, respectively as: A- homozygous tolerant, B- homozygous sensitive, AB – heterozygous and '–' missing for each wheat genotype. Single marker analysis was carried out by fitting the data on the SSRs (as independent variable) and the phenotypic data (as dependent variable) of 152 plants F_2 population using single linear regression model given as y = bo+b1x+e (Basten *et al*., 2000). The estimated genetic map of SSRs was used as a framework for the positioning of QTL using composite interval mapping (Zeng, 1994; Basten *et al*., 2000) by associating the values for different traits. The percentage of variation and additive effect of each of phenotypic traits caused by the presence of QTLs were also estimated using WinQTL Cartographer version-2.5.

RESULTS AND DISCUSSION

Analysis of variance for five check varieties and two parents conducted in two environments, E_1 and E_2 indicated significant variation due to genotypes for every trait studied except for chlorophyll fluorescence. Kumar *et al.* (2014) evaluated the 50 diverse wheat genotypes and exhibited highly significant differences at genotypic level for all the traits studied, under normal and heat-stress environments. All the traits expressed significant interaction with environments, indicating that all traits respond to high temperature in different ways in different genotypes. Talukdar *et al.* (2014) reported one of the first linkage maps in wheat using genotype by sequencing SNP markers to extreme response to post anthesis heat stress conditions and also evaluated that the molecular markers *Xbarc113* and AFLP AGCTCG-347 on chromosome 6A, *Xbarc121* and *Xbarc49* on 7A, *gwm18* and Bin1130 on 1B, Bin178 and Bin81 on 2B and Bin747 and Bin1546 on 1D were associated with these QTL. Analysis of variance, exhibited highly significant differences at genotypic level for all the traits studied, under normal and heat-stress environments. All the traits expressed significant interaction with environments, indicating that all traits respond to high temperature in different ways in different genotypes. This variability gives suf-

Table 1. DNA amplification profile of parental varieties of two wheat crosses using SSRs.

	PBW373 × WH1081
Number of markers used	200
Number of markers that did not show amplification	42
Number of markers that show amplification	158 (79%)
Number / percentage of amplified markers showing polymorphisum	22
Number of alleles detected using polymorphic markers	32
Range of alleles	1-3
Average number of alleles	1.45
Size of products	100-300 bp

Table 2. Association of heat stress and related traits and primers detected by single marker analysis using F_2 population of cross PBW373 × WH1081.

Traits	**Name of markers**	**Chromosome**	**Significance**
Days to heading	*Xbarc1044, Xgwm666.2, Xgwm635*	3A, 3A, 7A	*, *, *
Days to anthesis	*Xgwm635, Xgdm125*	7A, 4D	*, *
Days to physiological maturity	*Xwmc473*	4D	*
Grain filling duration	*Xbarc142, Xwmc473*	5A, 4D	*, *
Plant height (cm)	*Xbarc142, Xgwm337, Xwmc336*	5A, 1D, 1D	*, *, *
Number of productive tillers/ plant	*Xgwm2, Xgwm666.2*	3A, 3A	*, *
Number of grains/spike	*Xbarc142*	5A	*
1000 grain weight (g)	*Xbarc142, Xwmc473*	5A, 4D	*, *, *
Grain yield/plant (g)	*Xgwm2*	3A	*
Membrane thermostability (%)	*Xgwm156*	3B	*
Chlorophyll fluorescence (F_V/F_M)	*Xgwm611*	7B	*

Table 3. Distribution of markers over 16 chromosomes categorized as framework markers and their genetic lengths.

Linkage group	**SSRs**		**Length (cM)**
	Number of markers	**Name of markers**	
1A	1	*Xwmc336*	24.3 cM
2A	1	*Xwmc170*	131.3 cM
3A	4	*Xgwm2, Xgwm369, Xbarc1044, Xgwm666.2*	326.4 cM
4A	1	*Xwmc313*	181.0 cM
5A	2	*Xgwm293, Xbarc142*	207.5 cM
6A	2	*Xwmc398, Xbarc142*	190.9 cM
7A	2	*Xgwm635, Xgwm260*	296.0 cM
1B	1	*Xgwm413*	25.0 cM
3B	2	*Xgwm156 , Xbarc147*	102.8 cM
6B	1	*Xwmc398*	55.6 cM
7B	1	*Xgwm611*	89.0 cM
1D	2	*Xgwm337, Xwmc336*	47.2 cM
2D	2	*Xbarc142, Xwmc170*	274.1 cM
3D	2	*Xgdm8, Xcfd70*	131.5 cM
4D	2	*Xwmc473, Xgdm125*	386.2 Cm
7D	1	*Xgwm635*	80.5 cM
A genome	13		1360.3 Cm
B genome	5		54.48 Cm
D genome	9		919.5 Cm

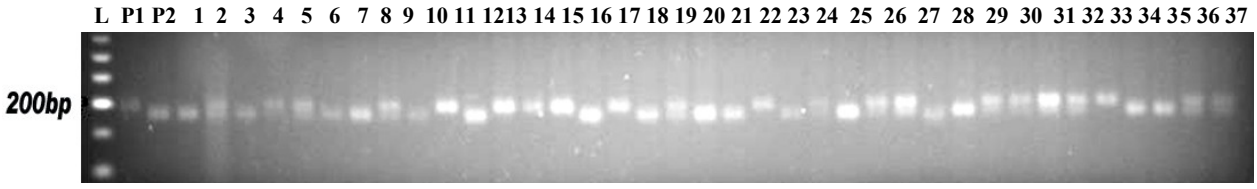

Fig. 1. *Agarose gel showing polymorphic bands of F_2 plants from cross PBW373 x WH1081 along with parental genotypes using SSRs Xgwm337.*

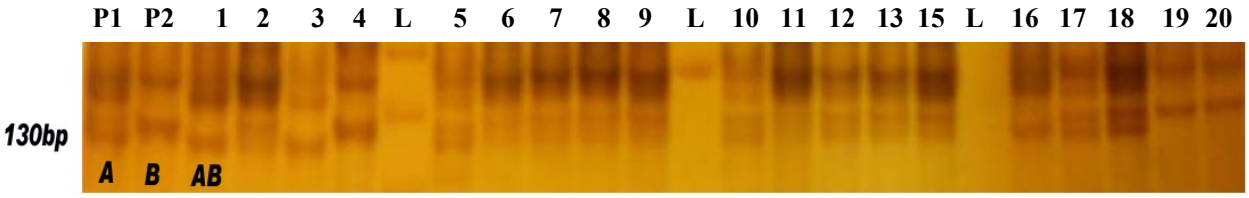

Fig. 2. *Polyacrylamide gel showing polymorphic bands of F_2 plants from cross PBW373 x WH1081 along with parental genotypes using SSRs Xbarc1044.*

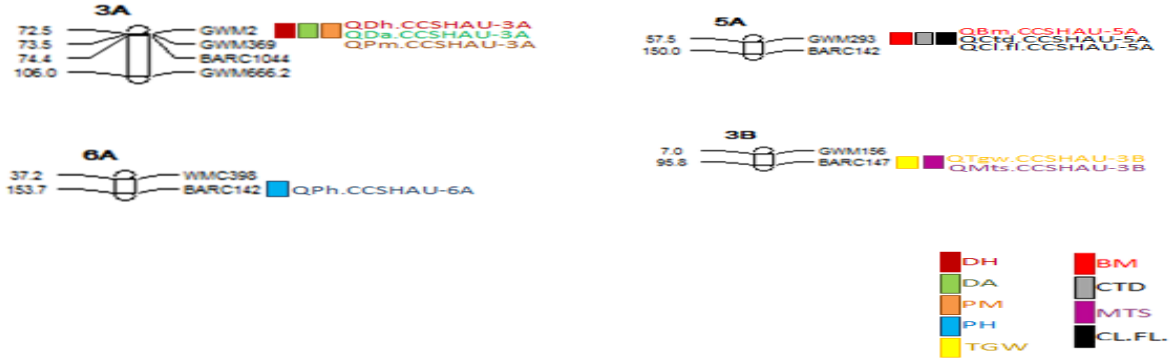

Fig 3. *Maps showing the location of 9 QTLs on different chromosomes for physiological, yield related traits using cross PBW373 × WH1081.*

ficient scope for further selection of the traits under consideration. F_2 population of 152 plants from viz; PBW373 (thermotolerant) × WH1081 (thermo sensitive) was phenotyped for heat stress related physiological, yield and its component traits. The population exhibited large variability for each trait.

Out of 200 SSRs used for amplification of parental DNA 158 SSRs (79%) showed amplification in parents out of which 22 SSRs (13.9% amplified SSRs) produced polymorphic bands. A total of 32 bands were observed. The number of alleles scored ranged from 1 to 3 with an average of 1.45 alleles (Table 1).

Association of QTLs with phenotypic traits (Single marker analysis, SMA): The single marker analysis allows the estimation of potential QTL by identifying markers segregating with phenotypic traits. This simple analysis was conducted with 22 SSRs to assess association of different traits with a marker using F_2 population. Three markers were detected to have association with QTL for days to heading (significant at 5% level). First two markers (*Xbarc1044, Xgwm666.2*) were mapped on 3A while one *Xgwm635* on 7A chromosome (Figs. 1 and 2). Two markers were detected to have association with QTL for days to anthesis (at 5% level significance) and these markers (*Xgwm635*and *Xgdm125*) were mapped on 7A and 4D chromosomes, respectively. One markers (*Xwmc473*) was detected to have significant association with QTL (at 5% level) for days to physiological maturity, mapped on 4D chromosome. Total two markers were detected to have significant association with QTL for grain filling duration at 5% level of significance. *Xbarc142* and *Xwmc473* markers were mapped on 5A and 4D chromosomes.

In F_2 population, three markers were detected to have association with QTL for plant height at 5% level significance. *Xbarc142* and *Xgwm337* markers were mapped on 5A while *Xwmc336* marker was mapped on 1D chromosome respectively. Two markers were detected to have association with QTL for number of productive tillers/plant with significance at 5% level. *Xgwm2* and *Xgwm666.2* markers were mapped on 3A chromosome. Only one marker (*Xbarc142*) was detected to have association for number of grains/spike with significance at 5% level and mapped on 5A chromosome. Two markers were detected to have association with QTL for 1000 grain weight at 5% level of significance while other was found to be associated with QTL at 0.1% significant level. *Xbarc142* and *Xwmc473* markers were found to be mapped on 5A and 4D chromosomes. One marker each *Xgwm2* was detected to have significant association with QTL for grain yield/plant (at 5% level) mapped on 3A chromosome, *Xgwm156* for membrane thermostability mapped on 3B chromosome and *Xgwm611* for chlorophyll fluorescence with significance at 5% level and mapped on 7B chromosome (Table 2). The result of regression analysis of each of the traits (i.e. plant height, number of productive tillers/plant, days to heading, days to anthesis, days to physiological maturity, grain filling duration, number of grains/spike, 1000 grain weight, grain yield/ plant, membrane thermostability and chlorophyll fluorescence) on individual markers was significant at 5% to 1% levels (Table 2). Yang *et al.* (2002) also detected two QTLs for heat tolerance measured by grain filling duration with the method of single factor analysis in an F_2 population. Pandey *et al.* (2013) screened Raj 4014 and WH 730 with different SSR markers. Out of 300 SSR markers

tested, 15% were found polymorphic. These polymorphic markers were utilized for genotyping a subset of RILs that had clear contrasting variation for difference in grain filling rate. To check for potential co-segregation of DNA fragments and heat tolerant phenotypes, simple regression analysis was carried out in order to confirm an association between the markers and the grain filling rate as indicator for heat tolerance. Out of the 35 markers tested, relationship between the two markers *Xbarc04* and *Xgwm314* and the phenotypes of RILs got established which were highly significant.

Composite interval mapping (CIM)

Construction of linkage maps: The 22 SSRs were used to construct the map by using the mapmaker. These were mapped on 16 linkage groups. The number of markers on these 16 linkage groups varied from one to four. One marker (*Xwmc336*) was found to be present on linkage groups 1A covering a length of 24.3 cM whereas linkage group 1B and 1D covered a length of 25.0 cM and 47.2 cM with one (*Xgwm413*) and two (*Xgwm337* and *Xwmc336*) markers respectively. Similarly 2A and 2D contained one (*Xwmc170*) and two (*Xbarc142* and *Xwmc170*) markers with overall chromosome length of 131.2 cM and 274.1 cM respectively. Four markers (*Xgwm2, Xgwm369, Xbarc1044* and *Xgwm666.2*) were found to be present on linkage group 3A, two markers (*Xgwm156, Xbar147* and *Xgdm8, Xcfd70*) each were present on 3B and 3D covered a total length of 326.4 cM, 102.8 cM and 131.5 cM. Linkage groups 4A and 4D covered a length of 181.0 cM and 386.2 cM with one (*Xwmc313*) and two (*Xwmc473*, *Xgdm125*) markers respectively. Two markers (*Xgwm293*, *Xbarc142*) were found to be present on linkage group 5A covered a length of 207.5 cM. On the otherhand chromosome 6A and 6B covered a length of 190.9 cM and 55.6 cM with two (*Xwmc398*, *Xbarc142*) and one marker respectively. Two markers (*Xgwm635*, *Xgwm260*) on 7A and one marker (*Xgwm611* and *Xgwm635*) each was found to be present on linkage group 7B and 7D with length of 296.0 cM, 89.0 cM and 80.5 cM. The total linkage coverage and average interval distance were 116.00 cM and 5.27 cM/Marker, respectively. The A-, B- and D- genomes had 1360.3 cM, 272.4 cM and 919.5 cM with average interval distance of 104.63 cM, 54.48 cM and 102.16 cM/ Marker. Partial genome maps were used in the present study and main effect QTL was detected by composite interval mapping using WinQTL Cartographer version-2.5. A logarithm of odds (LOD) score of 2.5 was used for suggesting the presence of a putative QTL.

Threshold LOD scores, calculated using 1000 permutations, were used for declaring definitive QTL. A total of nine QTLs were resolved following CIM (Table 3) of these, only one QTL was detected at a LOD score equal to threshold (2.5) value while eight were detected above the threshold value. The phenotypic variation explained by individual QTL ranged from 0.2% to 90.1%.

All the nine QTLs were having definitive located on chromosome 3A (QDh.CCSHAU-3A, QDa.CCSHAU-3A and QPm.CCSHAU-3A), cromosome 5A (QBm.CCSHAU-5A,QCtd.CCSHAU-5Aand QCl.fl.CCSHAU-5A), chromosome 6A (QPh.CCSHAU-6A) and chromosome 3B (QTgw.CCSHAU and QMts.CCSHAU-3B). Positive QTL effect suggested that an allele of the above QTL for heat stress tolerance is available in the tolerant parental genotype PBW373 (Fig. 3). Kumar *et al*. (2007) analyzed QTLs for grain weight (GW = 1000 grain weight) in common wheat using a set of 100 recombinant inbred lines (RILs) derived from a cross 'Rye Selection 111 (high GW) × Chinese Spring (low GW)'. Genotyping of RILs was done using 449 (30 SSRs, 299 AFLP and 120 SAMPL) polymorphic markers. QTL analysis for GW was conducted following genome-wide single marker regression analysis (SMA) and composite interval mapping (CIM) using molecular maps for the three chromosomes. Following SMA, 12 markers showed associations with GW, individual markers explaining 6.57% to 10.76% PV (phenotypic variation) for GW in individual environments. The CIM identified two stable and definitive QTLs, one each on chromosome arms 2BS and 7AS, which were also identified through SMA and a third suggestive QTL on 1AS. These QTLs explained 9.06% to 19.85% PV for GW in different environments. Paliwal *et al*. (2012) prepared a linkage map comprising 160 simple sequence repeat markers covering the whole genome of wheat. Using composite interval mapping, significant genomic regions on 2B, 7B and 7D were found to be associated with heat tolerance. Of these, two (2B and 7B) were co-localized QTL and explained more than 15% phenotypic variation for HSITGW, HSIGFD and CTD. The three major QTL obtained can be used in marker-assisted selection for heat stress in wheat.

Conclusion

Molecular markers are useful for breeders in selecting quantitative trait loci (QTL), where a trait has polygenic inheritance with variable heritability and needs to be selected in variable environments over generations. The objective of this study was to map and characterize quantitative trait loci controlling heat tolerance measured by different heat stress related physiological, yield and yield related traits in late sown conditions and to find the molecular markers associated with them. So the present study concluded that heat stress caused due to delayed sowing leads to reduction in mean performance of the varieties for almost all economic traits. However this reduction can be avoided to some extent by using thermo tolerant varieties. Breeding for such genotypes/varieties can be eased by identifying markers using molecular marker assisted selection.

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