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MOLECULAR MARKERS FOR GRAIN YIELD, YIELD TRAITS AND CANOPY TEMPERATURE IN SPRING WHEAT (*TRITICUM AESTIVUM* L.) UNDER LATE SOWN CONDITIONS OF EASTERN GANGETIC PLAINS OF INDIA

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ABSTRACT:

The heat stress is considered a major limitation to wheat productivity in arid, semiarid, tropical, and subtropical regions of the world, particularly the terminal heat stress which largely refers to rise in temperatures at the time of grain growth that can be a problem in up to 40% of irrigated wheat growing areas in the developing world. Increasing temperature caused by global warming is presently viewed as the greatest threat to wheat production in many countries including India. Current estimates indicate that in India alone around among 13.5 million hectares of wheat area is heat stressed. Therefore,



breeding for tolerance to high temperature stress is a top priority in India. The wheat yield has been found to generally decline as the temperature increases. The temperature stress intensity is severe particularly under late sowing conditions, causing reduction in the duration of later growth phases and thereby yield. Present study was initiated with the aim of investigating heat tolerance and identifying QTLs that are related to terminal heat stress on a Doubled-haploid population consisting of 140 lines (including parent) that were developed from the cross between Berkut (CIMMYT) and Krichauff (Australia). Sowing was done over three seasons 2007-2008, 2008-2009 and 2009-2010 under heat stressed environment in the eastern *Gangetic plains (EGP) of India. Total 233 markers were found to be polymorphic out of 1,150 SSR markers.* The linkage map was constructed using Map Manager QTX version QTXb20 by applying the Kosambi mapping function. The analysis of variance (ANOVA) of the DH population reflects that the mean square due to genotypes and environment for all trait were significant. The G*E interaction was found to be significantly affecting the traits studied. Using composite interval mapping 9 QTLs, for the traits yield ,thousand grain weight, grain filling duration, and canopy temperature for heat tolerance were detected on the chromosomes 2A, 2B, 4B, 5B, 6A and 7D. Among these, the QTL identified on the chromosome 7D was found consistently for three traits i.e., canopy temperature, yield and test weight. Two QTLs were found for yield (QLY.bhu-2A and QLY.bhu-7D), three QTLs were found for thousand grain weight (QLTgW.bhu-2B, QLTgW.bhu-6A and QLTgW.bhu-7D), only two QTLs could be found for grain filling duration (QLGfd.bhu-4B and QLGfd.bhu-5B, canopy temperature (QLCt.bhu-2B and QLCt.bhu-7D), the chromosomal location for one QTL was similar for CT, yield and TGW that was 7D whereas another QTL had similar location 2B for CT and TGW. The result of the QTL*Environment interaction was observed to be significant for all the traits observed in the study. The TGW and GFD QTLs QLTgW.bhu-6A and QLGfd.bhu-4B were robust QTLs with marker interval distance of 1.6cM and 2.2cM. Genomic regions for the three QTLs (QLY.bhu-7D, QLTgw.bhu-7D and QLCt.bhu-7D) were same in which one are co-localised QTLs for the trait TGW and CT. These QTLs can be used in marker assisted wheat breeding with the purpose of developing heat tolerant wheat varieties in future researches and would be considered a significant contribution for the future research and studies for yield related heat tolerance traits.

KEY WORDS: heat stress, future researches, wheat productivity.

INTRODUCTION:

Wheat (*Triticum aestivum* L.) is an important winter staple crop across the globe. However, due to continuous climatic change and global warming in many of the wheat producing countries there is concern of the reduction in the yield, that could be attributed to growth acceleration, reduction of the duration of phasic developmental stages and carbon starvation due to reduced net assimilation (Blum, 1988; Hall, 1992; Shpiler & Blum, 1991, Mohammadi *et.al* 2008). Over the last 50 years, wheat production has increased at the rate of nearly 1% per annum (Trethowan et al. 2002) and tolerance to high temperature stress has been identified as an important factor contributing to these increased yields (Assad and Paulsen, 2002). Heat stress is a major environmental stress limiting wheat productivity in most cereal growing areas of the world consequently making it the important breeding target in wheat.

Heat stress, particularly the terminal heat stress can be a problem in up to 40 percent of irrigated wheat growing areas in the developing world covering more than 36 m ha (Joshi *et al.*, 2007a). Significant part of wheat in South Asia is affected by heat stress and the current estimates indicate that in India alone, around 13.5 million ha area is heat stressed (Joshi et al., 2007b). Shorter crop duration and period of grain-filling and higher temperatures during crop growth, particularly during grain filling are among the reasons for the lower productivity of wheat in India. Beside other reasons, this could also be attributed both to the proximity to equator and predominant Rice-Wheat cropping systems, leading to delayed sowing of wheat that cause exposure to high temperatures particularly during grain filling period in India and other neighboring countries (Tandon, 1994; Rane et al., 2000). Grain filling duration has been widely used as a measurement of heat tolerance (Fokar et al., 1998). Therefore wheat breeding efforts are directed to address this problem in the subtropical wheat growing states of India with the target of increasing yield potential and attaining yield stability of wheat in marginal environments that remains a priority under increasing environmental instability created by the changing global climate (Reynolds *et al.*, 2007). Canopy temperature (CT) shows a strong and reliable association with yield under heat stress and is used in wheat breeding to select for yield, although little is known of its genetic control. The use of CT as a selection criterion to improve tolerance to heat was supported by its significant association with grain yield and the genotype differences observed between cultivars (Reynolds et al., 2001).

It has been observed that the heat stress occurring at the booting and heading, anthesis and grain filling stages causes floret sterility, abnormal pollination and ultimately results in low seed setting rate. As a winter crop, wheat has an optimal day time growing temperature during reproductive development of 15°C and for every 1°C above this optimum a reduction in yield of 3-4% has been observed (Wardlaw et al.1989). High temperature stress during reproductive development is particularly detrimental with post anthesis heat stress resulting in reduction in both individual kernel weight and and kernel number (Hays et al. 2007a; Plaut et. al. 2004; Randall and Moss 1990; Tashiro and Wardlaw 1990; Wardlaw and Wrigley 1994).

Information regarding the genetic basis of heat tolerance is scarce, though the use of traditional plant breeding protocols and contemporary molecular biological techniques, including molecular marker technology and genetic transformation, have resulted in genetic characterization and/or development of plants with improved heat tolerance. In particular, the application of quantitative trait locus (QTL) mapping has contributed to a better understanding of the genetic relationship among

tolerances to different stresses. It has been proved by earlier workers that heat tolerance is quantitatively inherited and continuously distributed (Blum 1988, Yang *et al.*, 2002). QTL mapping offers important information on the number and location of the loci that control quantitative traits and may provide a useful method for marker assisted selection (Liu 1998). Although wheat varieties showing improved yield stability under heat stress have been identified (Hays *et al.*, 2007a, b; Yang *et al.*, 2002c), the quantitative nature of heat tolerance and unpredictability of heat stress in the field makes it particularly difficult for breeders to effectively select for the trait and therefore breeding for heat tolerance needs intensive and enhanced efforts (Ortiz *et al.*, 2008; Ashraf, 2010). Quantitative trait loci analysis, correlation and co-segregation approaches, and the use of genetic stocks are most applicable to the dissection of the genetic basis of thermo tolerance in cereals (Holden *et al.*, 1993; Blum *et al.*, 2001).

With_the development_of molecular technology, it has become possible to detect the Quantitative trait loci (QTL) conferring heat tolerance in wheat for yield and analyze their genetic effects. The QTL to be detected could facilitate the understanding of genetic mechanisms of QTL cloning and Marker assisted selection in wheat breeding

In view of the above background about the heat stress in EGP and its related loss to wheat the objective of this study was to carry out a detailed characterization of heat stress under late sown condition and to find out the QTLs that are associated with the yield and related yield traits under heat stress condition due to late panting.

MATERIALS & METHODS

Plant material

The Doubled-haploid population consisting of 140 lines, used in the present study, were developed by South Australian Research and Development Institute, Australia derived from a cross between bread wheat Berkut (Irene/Babax//Pastor) from CIMMYT (2002) and Krichauff (Wariquam//Kloka/Pitic-62/3/Warimek/Halberd/4/3-Ag-3/Aroona) from Australia (1996) where the Berkut was used as heat sensitive parent while Krichauff was heat tolerant (Balouchi, 2010).

Field Management and data scoring

The experiments were planted at Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (25.18° N latitude; 83.03° E longitudes, 75.7 m above mean sea level, soil pH 7.4) which sits on the EGP of India. Invariably there is exposure to high temperatures at the end of the wheat season in the EGP of India (Joshi et al. 2007a) which is considered as a heat-prone mega-environment (ME), classified as ME5 (Braun et al. 1992). The experiments were planted in three consecutive seasons 2007-08, 2008-09 and 2009-10 under late sown (3rd week of December) to represent heat stressed environments. Daily temperatures given in Fig. 1 indicate that the late sown experiment was heat stressed compared to the normal sown.

All the plantings were conducted under irrigated conditions and four irrigations were applied during the entire experimental period. The trial was sown in a Randomized Complete Block Design with 6 row plots of 3 m length and 25 cm spacing between the rows (plot size 4.5 m²) in 3 replications. Each replication consisted of four blocks having 35 lines with a check (HUW 468) after every 5th line to serve as a covariate in the ANOVA analysis. The covariate values for each line were determined as the average of the checks lying on its two sides. At the beginnings/ends of the blocks, however, where the lines had the checks at only one side of them, the value of the check nearest to them was used.

Fertilizer was applied as per the recommendations for the EGP i.e., 120 kg of N, 60 kg of P_2O_5 and 40 kg of K_2O per hectare in each year. While P_2O_5 and K_2O were applied during sowing, the N application was split in 3 doses; during sowing and one each after the 1st and 2nd irrigations. The crop was protected from spot blotch and leaf rust (two of the most serious fungal diseases of wheat in EGP) by applying the fungicide Tilt (propiconazole); @ 625 g a.i./ha) at two growth stages GS 54 and 69 (Zadoks et al. 1974). The plots were kept weed free by applying pre-emergence herbicide

pendimethalin (@ 1000 g/ha in 500-600 liters of water/ha at 3 days after sowing) and sulfuron (@ 25.0 g/ha in 250-300 litres of water/ha was sprayed at 25 days after sowing).

The traits recorded were yield, thousand grain weight, canopy temperaure, date of anthesis and physiological maturity (75% of the plants mature per plot) for whole DH population and parental genotypes. The days to anthesis were subtracted from days to physiological maturity to obtain the GFD for each line.

The CT was recorded at three growth stages viz., heading (GS55), anthesis (GS65) and early dough (GS83) (Zadoks et al. 1974) on bright sunny days between 1200h and 1400h at 7 day intervals. For this measurement a hand held infra red thermometer (Mikron Infrared Inc. NJ, USA) was used holding at an angle of 30° to the horizontal plane, one meter away from the edge of the plot and approximately 50 cm above the crop, giving a canopy view of 10 cm × 25cm.

DNA EXTRACTION AND GENOTYPING

DNA extraction

DNA extraction of the two parental and 140 doubled haploid lines (including the two parents) of the Berkut/ Krichauff mapping population was carried following Williams *et al.*, (2006) with some modification as given in Genc et.al (2010).

Genotyping

Genotyping using SSRs was carried out using standard PCR conditions and subsequent gel electrophoretic separation on 8% polyacrylamide gels for identification of polymorphic markers as described by Genc et al (2010). The SSRs used have earlier been described: *barc* (Song et al. 2002; 2005), cfa (Sourdille et al. 2003), cfd (Guyomarc'h et al. 2002), gdm (Pestsova et al. 2000), gwm (Röder et al. 1998) and wmc (Gupta et al. 2002). Multiplexed PCR amplification and product separation were performed on an ABI3730 capillary sequencer (Applied Biosystems, Warrington, UK) as described by Hayden et al. (2008). The gel separation was used for identification of polymorphic markers and genotyping of mapping population for map construction whilst QTL analysis was done using an ABI3730 capillary sequencer. Subgenome-specific primers of *Vrn1* genes were used according to Fu et al. (2005). In addition to the 216 SSRs and Vrn genes, 311 DArT markers were mapped by Triticarte Pty Ltd. (http://www.tritcarte.com.au). Genomic DNA of the mapping population consisting of parents and DH lines was hybridized to the wheat DArT Version 2.0 containing 5,137 clones, and polymorphism detected and scored as described by Akbari et al. (2006). DArT markers consisted of the prefix `wPt` followed by number corresponding to a particular clone in the genomic representation, where w = wheat, P = *Pst1* (primary restriction enzyme used) and T = *Taq* (secondary restriction enzyme) (Jaccoud et al. 2001).

Linkage Map construction and QTL Detection

The linkage map was constructed using Map Manager QTX version QTXb20 (Manly et al. 2001) by applying the Kosambi mapping function with a threshold value, P = 0.01. Genotypic data from the DH population was initially arranged into groups via the "Make Linkage Groups". New markers were integrated into chromosomes using the "Links report" function, and then in conjunction with the "ripple" function and published maps an order of markers was established to minimize double recombinants and chromosome length. Marker order was verified using RECORD with ripples = 0, EQV threshold = 0 (Van Os et al. 2005). Segregation ratios of two genotype classes (Berkut allele and Krichauff allele) at each locus were tested using a chi-square test (P < 0.05). The segregation ratio at a locus deviating from the expected (1:1) indicated distorted segregation and was excluded from the QTL analysis.

QTL Cartographer version 2.5 (Wang et al. 2007) was used to find the QTLs by simple interval mapping (SIM) and composite interval mapping (CIM). The parameters settings for CIM were model 6, forward and backward stepwise regression with threshold of P <0.05 to select cofactors, window size 10 and 2 cM walking speed along chromosomes. QTLs were verified by LOD scores compared to an

empirical genome-wide significant threshold calculated from 1000 permutations for P <0.01 to control Type-I error. Adjusted mean of yield related traits for heat tolerance each year were calculated before pooling the data from all years. The QTL × QTL and QTL × Environments interactions were calculated using the program QTL Mapper 1.60 (Yang et al. 2005) http://ibi.zju.edu.cn/software) and Joint analysis was done with PlabQTLV1.2 software (Utz and Melchinger 2000 http://www.uni-hohenheim.de/*ipspwww/soft.html).

One thousand permutations were used to obtain thresholds (LOD of 2.46 at 0.05 significance level), for claiming significance of QTL. The QTLs were designated based on the nomenclature in the catalog for gene symbols for wheat International Rules of Genetic Nomenclature (http://wheat.pw.usda.gov/ggpages/wgc/98/), consisting of a "Q" followed by the trait name, institution designation, and chromosome assignment. QTL for HSI of GFD that co-localized within the same genomic region were assigned a common QTL name.

Statistical analysis-

Analysis of variance (ANOVA) as well as phenotypic correlation coefficient between the traits for three trials was performed using GenStat-12.1/2009 (www.vsni.co.uk/genstat) programme.

Heritability was estimated using following formula (Nyquist 1991):



where, h^2 represents the heritability, σ_g^2 is genetic variance, σ_{gt}^2 is genotype × trial variance, σ^2 is error variance, r is number of replications and t is the number of trials.

RESULTS-

Phenotypic analysis of DH

The analysis of variance (ANOVA) of the Berkut/Krichauff derived DH population(Table1) reflects that, the mean square due to genotypes and environment for yield, thousand grain weight, grain filling duration and canopy temperature were significant. The G*E interaction was found to be significantly affecting the traits studied. It was observed that there was continuous and almost normal phenotypic distribution for all the traits viz., Yield, TGW, GFD and CT in the DH population studied indicating the quantitative inheritance of all the characteristics. The t-test indicated significant differences for the traits between the parents over entire experimental years (p=0.001).

The yield value in kilogram/hectare (Table 2) were 1900.5, 2817.34 and 2722.4 during three years in case of Krichauff, however it was found to be 1771.3, 2380.6 and 1899 in Berkut The values of TGW in gram of heat tolerant parent Krichauff was 28.8 in 2007-08 while 37.7 and 28.1 in 2008-09 and 09-10; whereas the TGW in gram for the heat susceptible parent Berkut was 25.8, 34.6 and 24.2 during the same three years of study. The values of GFD in days for Krichauff were 26.3 (2007-08), 28.3 (2008-09) and 27.3 (2009-10) whereas for Berkut it were 22.3, 24.6 and 23.3.. The CT in degree celsius in tolerant parent were observed to be 30.3 (2007-08), 29.3 (08-09) and 28.7 (09-10) whereas in Berkut these were 33.6, 32.7 and 30.0

The mean values of the DH population for the traits during the consecutive three years of experimentation were observed as 1826.8, 2216.8 and 1883.9 kg/hec in three consecutive years for yield.The mean TGW in gram value were 23.9 (2007-08), 28.5 (2008-09) and 24.3 (2009-10). While for GFD the values (in days) were 25.3, 28.1 and 23.5 during the same period. Finally, for the CT the values in degree celsius were observed to be 32.8, 31.9 and 30.4 in the same three years of experiment (Table 2)

Varied heritability (Table 1) was found for all the four the traits. For yield 51%, for thousand grain weight the heritability was 63%, whereas for GFD it was found to be 62% and finally for CT it was 40%. The correlation for each trait across years was positive and significant (data not shown) indicating the experiments were able to provide proper trait expression. The correlation of Yield with TGW was positive and significant while with CT it was negative (data not shown).

QTL Analysis-

Of 1,150 SSR markers used to screen polymorphism between the DNA of Berkut and Krichauff, 233 were polymorphic. Among the 233 markers, 17 were unlinked and could not be assigned to any linkage group. Therefore, only 216 markers were used in the QTL mapping analysis

We found 9 QTLs for yield and yield related traits viz TGW,GFD,CT (,Fig 2a-I and Fig 3).

For yield two QTLs were found. The first yield QTL, QLY.bhu-2A was found on 2A chromosome between the markers *wpt1657* and *gwm275* with corresponding LOD values 2.5 and 2.34 in first and third year it was not found in the second year of testing, the corresponding R² values were 11.46 and 10.75. The mean values were 2.35 and 11.13%.(Table 3, Fig 2a) The second yield QTL QLY.bhu-7D was found on 7D chromosome detected over three years between the two markers gwm437 and wmc488a with corresponding LOD value 3.34, 3.59, 3.36 and R² values 15.45 %, 16.56% and 15.37% respectively with mean LOD value of 3.36 and R² value 15.45% (Table 3, Fig 2b). Three QTLs were found under late sown condition for thousand grain weight on the chromosome 2B, 6A and 7D respectively. The first QTL QLTgW.bhu-2B was on the chromosome 2B with interval size 9.2 cM and LOD values 5.72, 5.5 and 5.54 covering R² values 26.56%, 25.62% and 25.44% with mean LOD value 5.55 and R² value 25.78% (Table 3, Fig 2c). The second QTL *QLTgW.bhu*-6A was on the chromosome 6A between the markers *cfd080 –cfd080LH* with interval size 1.6cM in three years with LOD values 3.78, 3.45, 3.91covering R² values 20.52%, 16.34 and 18.82% and mean LOD values 3.43 and R² value 16.89% (Table 3,Fig 2d). While the third QTL, *QLTgW.bhu*-7D for thousand grain weight found on the chromosome 7D between the markers *wmc436b* and *barc214* with LOD values 2.86, 2.55 and 3.20 and R² value 13.11%, 11.82% and 14.75% the mean values were LOD 2.62 and R² 12.14% (Table 3, Fig 2e).

Two QTLs were found for grain filling duration one on chromosome 4B and another on 5B. The first QTL, *QLGfd.bhu*-4B was between *wpt6209- wpt7062* markers with interval size 2.2cM and LOD values 2.85, 1.97 and 2.87 in the all three years respectively while the variance was 13.23%, 8.76% and 13.23% respectively, the mean LOD value was 3.24 and the mean phenotypic variance was found to be 15.15% (Table 3,Fig 2f). Whereas the other QTL *QLGfd.bhu*-5B was observed on the chromosome 5B between the markers *wpt3457-wmc289* having the interval size 19.5cM and the LOD values of 3.20, 2.74, 3.25 with variance 14.93%, 12.58% and 14.93% respectively over the three years. The mean LOD and R² values where 2.95 and 13.56% (Table 3,Fig 2g). For canopy temperature under late sown condition we could find only 2 QTLs one on chromosome 2B and the other on 7D as incase of the trait yield, thousand grain weight. *QLCt.bhu*-2B explained 11.42%, 12.20%, 9.82% phenotypic variance for canopy temperature, it coverd LOD scores 2.47, 2.66 and 2.10 with mean LOD value 2.23 and R² value 10.39% (Table 3,Fig 2h). The second QTL *QLCt.bhu*-7D was found on chromosome 7D explaining 2.50, 2.27, 2.70 LOD values and 11.47%, 10.45% and 12.42% phenotypic variance with mean LOD and R² values 2.60 and 11.95% (Table 3,Fig 2i).

TheQTL *Environment interaction was found slightly significant for all QTLs (Table 3)

The QTL*QTL interactions was observed between the chromosome 2A,2B ,5B,6A and 7D but the QTL*QTL*Environment interactions were non significant for them The identified QTLs located on the Chromosome 2B and 7D showed significant interactions with the LOD value 3.05 in first year, The QTLs located on chromosome 2B and 5B as well as 5B and 6A showed significant interaction with LOD values 5.84 and 8.41 in the second year of testing while the QTLs located on 2A and 2B showed significant interaction with LOD value of 5.35 in third year (Table 3)..

In the joint analysis (Table 3) for QTL of yield maximum phenotypic variance explained 36.9% with LOD value 16.01 in the second year 2008-09 while in the first year 2007-08 it were 34.6% and 14.74 whereas in the third year 2009-10 phenotypic variance was down to 23.4% with LOD value 13.6 The joint analysis mean over year for yield was 36.8% with LOD value of 15.95. The joint analysis for

the QTLs of thousand grain weight maximum phenotypic variance was 58.1% with LOD value 27.64 in the first year 2007-08 while in the second and third year 2008-09 & 09-10 it were slightly down to 56% &26.77 and 55.5% & 26.17 respectively whereas the mean over year for TGW was 55.8% &26.15. The joint analysis for the QTLs of GFD explained phenotypic variance 46.5% and LOD value 21.30 both in the first and third year of experiment while it was down in the second year to 37.6% and 16.12 while the mean over the years was 38.6% & 16.57. The joint analysis for QTLs of canopy temperature explained 9.7% variance and LOD value 3.36 in 2007-08 which were observed to be 10.9% & 3.76 in second year 2008-09 and still higher in the third year of experiment with variance 16.5% and 6.06 with mean over the years values for variance and LOD 17.4% & 6.59 (Table 3).

We found QTL on Chromosome 7D for Yield ,TGW ,and CT with the same flanking markers (wmc436b-barc214) Thus the QTL s was co-localized with that of TGW and CT. Likewise one of the markers (ta0232) on chromosome 2B was similar for the QTLs of TGW and CT.

DISCUSSION

DH population's phenotypic analysis

The significant mean square due to genotypes and environment for canopy temperature, grain filling duration, yield and thousand grain weight as found in the result (ANOVA) suggests the existence of considerable variation among the genotypes, beside for the traits for which they were evaluated and environment. Significant Genotype*Environment interaction was found for all the traits when tested against pooled error, thereby indicating that the traits were influenced by environmental changes.

The significant variation in the result supports the finding of normal distribution leading to the finding of QTLs that are associated with heat tolerance in the present study. The normal distribution of the traits studied among the genotypes reflects their quantitative nature. The quantitative nature of heat tolerance has been shown earlier also by many workers in view of the genetic diversity and heat stress tolerance (Amudha and Balasubramani, 2010;Midmore *et al.*, 1984; Rawson, 1986; Blum, 1988; Wardlaw *et al.*, 1989; Al-Khatib and Paulsen, 1990; Reynolds *et al.*, 1994).

The parents, Berkut and Krichauff as well as all the DH lines, showed phenotypic variation for the traits TGW, GFD, Yield and CT, in the first year of experiments subsequently targeting them for analysis during next two years of experimentation. These lines possessed the potential to affect the productivity and heat tolerance capacity besides having the variation and therefore their by inclusion in study was justified. We observed that mean values were invariably consistent for most of the traits throughout the three years. Minimal and non- significant reduction was observed in Krichauff (tolerant) under heat stress for all the traits during three years, this finding reaffirms observations of Mason *et al.*, 2010, in whose experiments the heat tolerant parent (Halberd) showed minimal reduction under heat stress for yield related traits. Estimated broad sense heritability for Yield,TGW ,GFD and CT were found to be 51%, 63%, 62% and 40% respectively.. The high heritable traits viz., yield and thousand grain weight are considered desirable as selection criterion for the heat stress tolerance. It was interesting to note that there was QTL*QTL interaction between the yield and TGW which makes these traits together as a desirable selection parameter.

Heritability of GFD was 62% in the present study while some earlier workers obtained higher heritability (93%, 80%, 96%, 89%, 76%; Barkat *et al.*,2011; Yang *et al.*,2002; Fokar *et al.*,1998, Riaz-ud-din et. al., 2010, Khan and Naqvi, 2011). However it is interesting to note that these results of other workers were obtained under controlled condition, while our results were from the experiments conducted in the natural experimental fields.

The high value of broad sense heritability for yield and thousand grain weight observed in our study (51 and 63%) are similar to some previous reports for grain yield (74-96%) medium (31-51%) for thousand grain weight and grain yield (Khan et al 2007; Rashidi, 2011; Riaz-ud-din et. al., 2010). The heritability of CT in the present study was in accordance with the results of Reynolds *et al.*, 2001, who reported moderate to high heritability. G*E was significant for most traits in three treatments. Yang *et al.*, (2002) determined minimum 1.4 genes with both additive and dominance effects, broad-sense heritability of 80%, and realized heritability of 96% for GFD from F_2 and F_3 populations.

QTL Analysis

Following CIM QTL analysis, total nine QTLs were detected which were two each for yield (*QLY.bhu-*7D, *QLY.bhu-*2A), three for TGW (*QLTgW.bhu-*7D, *QLTgW.bhu-*6A, *QLTgW.bhu-*2B), and, two GFD (*QLGfd.bhu-*4B, *QLGfd.bhu-*5B) and two for CT (*QLCt.bhu-*2B, *QLCt.bhu-*7D). The QTLs that were found for observed traits were related to heat stress owing to positive performance under heat stress natural late sown condition that resulted in finding these. Three QTLs (*QLCt.bhu-*7D, *QLY.bhu-*7D, *QLTgW.bhu-*7D) were detected on the same chromosome locations in three years, these were for CT, Yield and TGW on chromosome 7D; for TGW and CT QTLs the flanking markers (*wmc436b-bacr214*) were same, these QTLs were established as colocalized,.Similarly, the QTLs for CT and TGW at chromosome 2B (*QLTgW.bhu-*2B and *QLCt.bhu-*2B) had one similar marker *ta0232* and therefore they are also established as being important information. The range of phenotypic variance for all identified QTL was 8.76 to 58.1.

For yield two QTLs *QLY.bhu*-7D, *QLY.bhu*-2A, were found on chromosome 7D and 2A between the markers *gwm437-wmc 488a* and *wpt1657-gwm275*, with LOD and R² values more than 3.3; 15.37% and 2.3; 10.75 respectively, with mean LOD value 3.36, 2.35 and R² 115.45, 11.13%. QTLs *QLCt.bhu*-2B, *QLsCt.bhu*-7D for canopy temperature under late sown were found on chromosomes 2B and 7D, exhibiting more than 9.32 and 10.45% phenotypic variance, covering LOD scores more than 2.1 and 2.2. Similar to our findings Pinto *et al.*, 2010, also found QTLs for CT on chromosome 2B with phenotypic variance of 11.9% at vegetative phase (CTv) and 5.9% at grain filling stage (CTg); flanking markers for both were (aac/cta-1-agg/cta-3 and wpt9668-gwm388) while the markers of maximum effect were acc/ctg-4 (for CTv a=26.94) and gwm388 (for CTg a=45.59), it was also reported by them that the same markers were also found for NDVIg (normalized difference vegetative index at grain filling stage). The marker in our study (*tao232*) was common for both the trait TGW and CT.

Many workers have reported that traits such as CT are rapid and integrative in nature when considering the heat stress and are increasingly recognized not only as useful selection tools in breeding (Araus et al. 2008) but as valuable screens for high throughput phenotyping of mapping populations for abiotic stresses (Montes et al. 2007; Olivares-Villegas *et al.*, 2008).

Reynolds *et al.*, (1994 and 2001), have utilized the CTD and thousand grain weight as a selection criterion for tolerance to high temperature stress in wheat breeding Reports on cool canopy during grain filling period in wheat as an important physiological principle for high temperature stress tolerance are available beside CTD found higher and positive under a well irrigated wheat (Munjal and Rana, 2003; Bilge *et al.*, 2008). Vijailakshmi *et al.*, 2011 reported QTLs under high temperature that were related to senescence and found on the chromosomes 2A the same chromosome in our study was reported for yield though the markers were different. The chromosomes 7D, 4B, 6A 2A as reported by them for senescence related traits under heat stress were also same as in our case where the 7D was for CT, Yld and TGW, 4B for GFD, 6A for TGW and 2A for yield. In our study too the markers were different.

In the present study QTLs were found for CT, TGW and Yield on same location 7D in which TGW and Yield QTLs have same flanking markers (*wmc436b-barc214*), while for CT and TGW there was another similar chromosomal location 2B with one same marker tao 232, similar to these in an earlier study Pinto *et al.*,2010, in Seri/Babx population, also found that the QTL for both yield and CT co-localized on 4A-a. The variation under heat stress for yield were 11.13 and 15.45% while for the CT it was found to be 10.39% and 11.95%. Araki *et al.*,1999 also found a yield QTL on 4AL although under drought conditions. The canopy temperature depression (CTD) besides being a function of stomatal conductance (Amani *et al.*, 1996), is also a mechanism of heat escape (Cornish *et al.*,1991). Munjal and Rana (2003), have reported that cool canopy during grain filling period in wheat is an important physiological principle for high temperature stress tolerance. It can be concluded that canopy temperature can be used as a tool in the selection of wheat targeted for tolerance to heat stress. Panozzo *et. al.* 1999, also in a study emphasized the importance of cooler maximum spike temperature under field conditions.

In earlier studies five Yield and yield related QTLs have earlier been found having LOD scores greater than 3.0 and 4.5 to 17.1% increase in yield and yield components with phenotypic variance 9-21% (Huang *et al.*, 2003; Cuthbert *et al.*,2008; Felix Marza-Mamani, 2005; Groos *et al.*, 2003).

Present study lead to finding of 2 QTLs for the grain filling duration on chromosomes 4B and 5B. The first QTL, *QLGfd.bhu*-4B was between the markers wpt6209-wpt7062 (2.2cM) and LOD values more than 1.9, variance more than 8.7% while the second QTL *QLGfd.bhu*-5B had more than 12.5% phenotypic variance and was between *wpt3457-wmc 289* (19.5cM), the LOD values greater than 2.74.

There are earlier report of finding QTLs for GFD (Yang *et al.*, 2002, 2 QTLs on 1BS and 5AS with 11 and 12% phenotypic variation; Mason *et al.*, 2010, 5 QTL on 1B, 2A and 2D under heat stress with variance 9-15 %; Barakat *et al.*,2011, 3 SSR markers on 6A, 6B and 7D, linked to grain filling rate, accounting for phenotypic variance 3-25%). Our findings and results along with the above findings of earlier workers lead to consequence that the heat tolerance in common wheat is controlled by multiple genes.

Gross *et al.*, 2003, found 5AL to be associated with high temperature effect during grain filling on grain protein content. Susceptible varieties had up to 28.3% reduction for individual yield components. In the present study the heat tolerant cultivar Krichauff showed minimal reduction of yield component over three years.

Three QTLs were observed for TGW (on 2B, 6A and 7D). There was similar chromosomal location of the first QTL, *QLTgW.bhu*-7D along with the QTLs for yield and CT (*QLCt.bhu*-7D, *QLY.bhu*-7D, *QLTgW.bhu*-7D), having LOD value more than 2.55 (33.5 cM) covering R² value greater than 11.82. The earlier studies have reports on QTLs for kernel weight (1QTLHSI on 3B with R² 21% Mason *et al.*, 2010; 3 major QTLs for heat tolerance through SSI, on 1B, 5B and 7B LOD and R² more than 3 and 27.3% respectively, Mohammadi *et al.*, 2008; 3 QTLs on 2B, 5B and 7A Groos *et al.*,2003). The TGW can be better utilized for yield improvement owing to its repeatability across environments and accuracy of detection in comparison to the QTL for yield (Gross *et al.*,2002).

The joint analysis of segregation of marker genotypes, phenotypic values of individuals or lines, makes it possible to detect and locate the loci affecting quantitative traits (quantitative trait loci or QTL). The QTL analysis involves selecting and hybridizing parental lines that differ in one or more quantitative traits and analysing the segregating progeny so as to link the quantitative trait locus to known DNA markers. Kumar et al., (2009) performed joint analysis for spot blotch QTLs in wheat and reported multiple QTLs; in our study also we found multiple QTLs for TGW, yield, CT as well as GFD. Three years of precision evaluation of data for CT, GFD, Yield and TGW in the DH population lead to obtain resistance to heat stress under field condition, although the G*E interactions were significant for some traits. QTL*QTL additive interactions resulted in enhanced level of resistance for heat for GFD and thousand grain weight . Significant QTL*QTL interactions (chromosomes 2B and 7D in 1st year's LOD value 3.05 along; 2B and 5B, 5B and 6A in 2nd year with LOD values 5.84 and 8.41; 2A and 2B in 3rd year with LOD value 5.35 all these were with non-significant Q*E interaction) found in the present study is considered a significant contribution for the future wheat research since all these QTLs were in some way or the other interacting with each other and for this reason these traits in case of MAS can be used together as selection criterion. The epistatic effects and G*E interactions were detected to further understand the relations between loci, and how best to manipulate these relations so they could serve more useful for the purpose of heat tolerance breeding on the gene level. Successful MAS and cloning of the major heat resistance QTL in the future will crucially depend upon the generation of new flanking markers and the development of high resolution mapping populations and therefore it is expected that the QTL mapping achieved in this study would provide preliminary information to generate a finer map and initiate a marker assisted selection strategy. In addition, before the application of epistatic QTLs on MAS it is required to find out its increasing or decreasing effects on heat tolerance for a meaningful breeding utilization. The presence of epistatic interaction for the traits studied in the experiment (CT, GFD, Yield and TGW) may explain the occurrence of transgressive segregants in the DH population (Kumar et al. 2009b, Mohan et al., 2009).

According to the results obtained in the present experiment, the introgression study of favorable allele related to heat tolerance from the resistant parent will be carried out in the future researches.

Significant QTL*Environment effect was observed for all the traits. (Martin *et al.*, 2004) studied QTL*Environment interaction for kernel number in winter wheat with environment characterization based on probe genotype however. Environmental characterization has been shown to aid interpretation of Genotype*Environment interaction (Patterson *et al.*,1991; Stuber *et al.*,1992; Koester *et al.*,1993; Jansen *et al.*,1995; Ragot *et al.*,1995; Tinker *et al.*,1996; Monforte *et al.*,1997b; Brummer *et al.*,1997; Sari-Gorla *et al.*,1997; Melchinger *et al.*,1998; Hemamalini *et al.*,2000), this effect can be attributed to variation in field under different environment as evidenced by the ANOVA reflecting the interaction in the 9 QTLs mentioned earlier. However these significant effects were found to be moderate in general when compared to the main effect over the 3 years experimentation.

Besides the heat stress related QTLs found in the present study from the Berkut/Krichauff derived DH population , earlier workers have also reported several other QTLs from the same population that are related to different traits associated with salinity stress, grain fructan concentration and grain Arabinoxylan concentration (Table 4).

Co-localization of QTL with previous QTL studies:

The result of the study reflects that, three QTLs that were for the Yield, TGW and CT (*QLCt.bhu*-7D, *QLTgW.bhu*-7D) were found to be located on similar chromosomal location that is 7D, where the flanking markers for the QTLs of TGW(*QLTgw.bhu*-2B) and CT (*QLCtbhu*-2B) were same (*wmc* 436b-barc214). We also found that one marker ta0232 was common for TGW and CT QTL which had QTLs on similar chromosomal location 2B. Therefore on the basis of the above facts for the QTLs on 7D for TGW and CT, it is postulated that the QTLs are colocalised QTLs. Earlier reports of Mason *et al.*, 2010 and Mohammadi *et al.*, 2008, who detected a major QTL on 2D albeit the reported markers were different in both the reports including ours. Mason *et al.*, 2010 in their study found that the HSIGFD and HSI Kernel weight QTLs were co localizing on 1B and 5A with the one reported by Yang *et al.*, 2002c.

Stable QTLs

All the 9 QTLs found during the study were observed over three years' period of the experiment (except for the yield QTL *QLY.bhu*-2A, which was not seen 2nd year of study) and therefore were categorized as stable QTLs. The QTL*Environmental interaction for these QTLs was found to be slightly significant and thereby establishing these as stable ones, which could be a preferable trait for the future research.

CONCLUSION:

A genetic map for heat tolerance was generated in which 9 QTLs were observed for yield and yield traits in Berkut/Krichauff derived wheat DH population. The TGW and GFD QTLs *QLTgW.bhu*-6A and *QLGfd.bhu*-4B were robust QTLs with marker interval distance of 1.6cM and 2.2cM. These QTLs can be used in marker assisted wheat breeding with the purpose of developing heat tolerant wheat varieties in future researches.

	haploid lines derived from the cross Berkut x Krichauff under heat stressed condition								
	Source of Variation	df	YLD	TGW	GFD	СТ			
	Years	2	18625132*	2704.24**	2371.65**	674.36**			
	Rep (Years)	6	231518**	10.21**	23.25**	14.79*			
	Lines	139	381769**	50.15**	16.69**	1.96			
	Years x Lines	278	186921**	18.33**	6.37**	1.18**			
	Residual	834	35093	2.49	1.04	0.34			
Herita	bility % (Heat Stress very late sown)		51	63	62	40			

Table 1: Analysis of variance (ANOVA) for yield, thousand grain weight, grain fill duration and canony temperature for wheat double

*Significant at P = 0.05, **Significant at P = 0.01 YLD= Yield; TGW= Thousand grain weight; GFD= Grain filling duration; CT= Canopy temperature

Table 2: Distribution of the values of Yield ,TGW,GFD and CT of parents and 138 double haploid lines derived from the cross Berkut (heat susceptible)×Krichauff (heat tolerant)) under heat stress condition in three years (2007-2008, 2008-2009 and 2009-2010)

Geno type	Yield(kg /hec)			TGW(gram)			GFD (days)			CT(degree celsius)		
type	000-	0000	0000	000	200	200	200	200	200	200	000	200
Year	2007-	2008-	2009-	200	200	200	200	200	200	200	200	200
	08	09	10	7-08	8-09	9-10	7-08	8-09	9-10	7-08	8-09	9-10
Berk	1771.3	2380.6	1899±	25.8	34.6	24.2	22.3	24.6	23.3	33.6	32.7	30.0
ut	±9.4	±192.8	46.3	±0.4	±0.8	±0.1	±1.2	±0.3	±1.2	±0.3	±0.3	±
												0.00
Krich	1900.5	2817.3	2722.4	28.8	37.7	28.1	26.3	28.3	27.3	30.3	29.3	28.7
auff	±30.8	4±53.0	±44.2	±0.4	±0.1	±	±	±	± 0.3	±0.3	±0.3	± 0.6
						0.5	0.3	0.33				
DH	1826.8	2216.8	1883.9	23.9	28.5	24.3	25.3	28.1	23.5	32.8	31.9	30.4
mean	±28.2	±30.2	±28.2	±0.2	±-0.2	±0.3	±	±0.2	±0.2	±0.1	± 0.1	±.0.5
							02					

±Indicate standard error

MOLECULAR MARKERS FOR GRAIN YIELD, YIELD TRAITS AND CANOPY TEMPERATURE

VOLUME - 11 | ISSUE - 9 | JUNE- 2022

Table: 3 Effects of Quantitative trait loci (QTLs) that reduce heat stress in Berkut/Krichauff DH population detected by composite interval mapping (CIM) using QTL cartographer software (Wang et al. 2007)

Trait/QTLs	Marker Interval	Interval size (cM)) Chromo) some –	2007-2008		2008-2009		2009-2010		Mean over year		
				LOD	\mathbb{R}^2	LOD	\mathbb{R}^2	LOD	\mathbb{R}^2	LOD	\mathbb{R}^2	(Q×E)
Yield late sown												
QLY.bhu-7D	gwm437-wmc488a	6.9	7D	3.34	15.45	3.59	16.56	3.36	15.37	3.36	15.45	*
<i>QLY.bhu-2A</i> Joint analysis	wpt1657-gwm275	19.2	2A	2.50 14.74	11.46 34.6	16.01	36.9	2.34 13.6	10.75 23.4	2.35 15.95	11.13 36.8	*
Test grain weight	late sown											
QLTgw.bhu-7D	wmc436b-barc214	33.50	7D	2.86	13.11	2.55	11.82	3.20	14.75	2.62	12.14	*
QLTgw.bhu-6A	cfd080-cfd80-LH	1.6	6A	3.78	20.52	3.45	16.34	3.91	18.82	3.43	16.89	*
QLTgw.bhu-2B	tao232-wpt1140	9.2	2B	5.72	26.56	5.50	25.62	5.54	25.44	5.55	25.78	*
Joint analysis				27.64	58.1	26.77	56.0	26.17	55.5	26.15	55.8	
GFD late sown												
QLGFD.bhu-4B	wpt6209-wpt7062	2.2	4B	2.85	13.23	1.97	8.76	2.87	13.23	3.24	15.15	*
QLGFD.bhu-5B	wpt3457-wmc289	19.5	5B	3.20	14.93	2.74	12.58	3.25	14.93	2.95	13.56	*
Joint analysis				21.30	46.5	16.12	37.6	21.30	46.5	16.57	38.6	
CT late sown												
QLCt.bhu-2B	cfa2278-tao232	14.9	2B	2.47	11.42	2.66	12.20	2.10	9.82	2.23	10.39	*
QLCt.bhu-7D	wmc436b-barc214	33.5	7D	2.50	11.47	2.27	10.45	2.70	12.42	2.60	11.95	*
Joint analysis				3.36	9.7	3.76	10.9	6.06	16.5	6.59	17.4	
QTLx QTL inter	action 2B x 7D 2B x 5B 5B x 6A			3.05**		5.84** 8.41**						Ns Ns Ns
	2A x 2B							5.35**				Ns

 R^2 = total percentage of phenotypic variance explained by each QTL; *Significant at 5% level of significance; ns = non significant

Table 4: Description of different QTL found in Berkut/Krichauff Double haploid wheat
nonulation

S.N.	QTL	Chromosome	Nearest marker	Position (cM)	F- value	Trait	Reference
1	QGfc.aww- 2B.1	2B	barc91-2B	89.6	13.5	Grain fructan Conc.	Huynh (2008) Ph.D. Thesis
2	QGfc.aww- 2B.2	2B	wPt-7161-2b	208.7	9.5	u	u
3	QGfc.aww- 3B.1	3B	gwm802-3B	82.9	8.4	u	u
4	QGfc.aww- 6D.2	6D	barc54-6D	58.6	32.2	u	u
5	QGfc.aww- 7A.1	7A	gwm681-7A	5.4	54.8	u	u
6	Q.ls1A	1A	gbm1153/barc028a			Leaf symptoms	Genc et al. TAG,2010
7	Q.ls2D	2D	gwm102/wmc027			u	u
8	Q.ls5A	5A	gwm304/gwm186			u	u
9	Q.ls6D	6D	cfd287/cfd076a			u	"
10	Q.ls7A	7A	wPt-8897/wpt4784			u	"
11	Q.ls7A	7A	wPt5153/ksm019			u	"
12	Q.tn1A	1A	barc0281/gwm164			Tiller numbers	u
13	Q.tn4B	4B	wPt7082/gwm6			u	u

MOLECULAR MARKERS FOR GRAIN YIELD, YIELD TRAITS AND CANOPY TEMPERATURE VOLUME - 11 | ISSUE - 9 | JUNE- 2022

14	Q.tn5A	5A	wPt1370/Vrn1A			u	u
15	Q.tn5B	5B	Vrn1B/wPt5896			u	u
16	Q.tn5D	5D	cfd19a/Vrn1D			"	u
17	Q.sb2A	2A	gwm095/cfa2263			Seedling	
						biomass	
18	Q.sb4B	4B	wPt-7162/gwm6			u	"
19	Q.sb5A	5A	wPt1370/Vrn1A			u	"
20	Q.sb5B	5B	gwm213/wPt-0100			u	"
21	Q.sb6A	6A	cfd080/barc171			u	"
22	Q.sb6D	6D	cfd287/cfd076a			u	"
23	Q.sb7A	7A	gwm382/wPt0961			u	u
24	Q.ch2D	2D	gbm1209/wPt-0298			Chlorophyll	"
						content	
25	Q.ch5A	5A	wPt-1370/Vrn1A			"	u
26	Q.ch5B	5B	Vrn1B/wPt-5896			"	u
27	Q.ch5D	5D	cfd19a/Vrn1D			"	u
28	Q.mat5A	5A	wPt-1370/Vrn1A			Maturity	"
29	Q.mat5B	5B	wmc289/Vrn1B			"	u
30	Q.mat5D	5D	cfsd19a/Vrn1D			"	u
31	Q.Na2A	2A	wPt3114/wmc170			Na+	u
32	Q.Na2B1	2B1	wmc272/barc349			"	u
33	Q.Na2B2	2B2	wPt-7859/wPt7161			"	u
34	Q.Na6A	6A	cfd080/barc171			"	u
35	Q.Na7A	7A	wPt4744/gwm242			"	u
36	Q.K1D	1D	wPt4647/wmc147			K+	u
37	<i>Q.K3B</i>	3B	gwm299/gwm247			u	"
38	Q.K3D	3D	cfd223/cfd152			u	u
39	Q.K4A	4A	wPt7919/wPt0150			u	u
40	Q.K4D	4D	gpw95001/gwm165b			u	"
41	<i>Q.K5A</i>	5A	wPt1370/Vrn1A			"	u
42	<i>Q.K5B</i>	5B	Vrn1B/wPt5896			"	u
43	Q.K5D	5D	cfd19a/gwm292a			"	u
44	<i>Q.K7A</i>	7A	wPt-5153/ksm019			"	u
45	<i>Q.K7D</i>	7D	wPt2258/wPt3923			"	u
46	QGax.aww-	2A	wpt-3114-2A	85.7	22.4	Arabinoxylan	Nguyen et
	2A.1					conc.	al. Crop Sc. 2011
47	QGax.aww-	3D	wpt-0485-3D	0.0	9.9		
	3D.1						
48	QGax.aww-	4D	gpw-95001-4D	46.0	37.7		
	4D.1						
49	QGax.aww- 6B.1	6B	gwm680-6B	112.4	9.2		



Figure 1: Daily mean temperatures of the three wheat growing seasons (2007-08, 2008-09 and 2009-10) at Varanasi, India



MOLECULAR MARKERS FOR GRAIN YIELD, YIELD TRAITS AND CANOPY TEMPERATURE

VOLUME - 11 | ISSUE - 9 | JUNE- 2022



Fig. 2a-i: Likelihood of Odd Ratio (LOD) curves obtained by composite interval mapping for quantitative trait loci (QTL) mapped on chromosomes 2A,2B,4B,5B,6A and 7D for yield, thousand grain weight , grain fill duration and canopy temperature values over three years (2007-08, 2008-09 and 2009-10) of testing of 138 double haploid lines derived from the cross Berkut (heat susceptible) × Krichauff (heat tolerant) of wheat at Varanasi, India. Vertical lines indicate the threshold LOD value (2.46) determining significant QTL. Short arm on the top and open triangles indicate the probable position of centromeres

MOLECULAR MARKERS FOR GRAIN YIELD, YIELD TRAITS AND CANOPY TEMPERATURE



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