

# QTL mapping of terminal heat tolerance in hexaploid wheat (*T. aestivum* L.)

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**Abstract** High temperature (>30 °C) at the time of grain filling is one of the major causes of yield reduction in wheat in many parts of the world, especially in tropical countries. To identify quantitative trait loci (QTL) for heat tolerance under terminal heat stress, a set of 148 recombinant inbred lines was developed by crossing a heat-tolerant hexaploid wheat (*Triticum aestivum* L.) cultivar (NW1014) and a heat-susceptible (HUW468) cultivar. The F<sub>5</sub>, F<sub>6</sub>, and F<sub>7</sub> generations were evaluated in two different sowing dates under field conditions for 2 years. Using the trait values from controlled and stressed trials, four different traits (1) heat

susceptibility index (HSI) of thousand grain weight (HSITGW); (2) HSI of grain fill duration (HSIGFD); (3) HSI of grain yield (HSIYLD); and (4) canopy temperature depression (CTD) were used to determine heat tolerance. Days to maturity was also investigated. A linkage map comprising 160 simple sequence repeat markers was prepared 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. In pooled analysis over three trials, QTL explained phenotypic variation ranging from 9.78 to 20.34 %. No QTL × trial interaction was detected for the identified QTL. The three major QTL obtained can be used in marker-assisted selection for heat stress in wheat.

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## Introduction

Wheat is one of the most broadly adapted cereals. It is cultivated in a large area in the subtropics under continual heat stress, defined as having mean daily temperature greater than 17.5 °C in the coolest month of the growing cycle (Fischer and Byerlee 1991). Terminal heat stress largely refers to a rise in temperature at the time of grain growth (grain filling duration, GFD). High temperature during grain development (grain filling) is a major limitation to wheat production in many environments worldwide (Hays et al. 2007). Heat stress is a problem in 40 % of temperate environments which covers more than 36 million ha (Reynolds et al. 2001). A significant portion of the wheat grown in South Asia is considered to be affected by heat stress, of which the majority is present in India (Joshi et al. 2007a). The most heat-stressed locations of

South Asia are the Eastern Gangetic Plains (EGP), central and peninsular India and Bangladesh, whereas heat stress is considered moderate in northwestern parts of the Indian Gangetic Plains (IGP) (Joshi et al. 2007b; Singh et al. 2007).

Transitory or constantly high temperatures cause an array of morpho-anatomical, physiological and biochemical changes in plants, which affect plant growth and development and may lead to a drastic reduction in yield. In wheat, high temperatures (>30 °C) after anthesis can decrease the rate of grain filling (Stone and Nicolas 1995), while high temperatures imposed before anthesis can also decrease grain yield. Yields are reduced 3–4 % per 1 °C rise above the optimum temperature (15–20 °C) during grain filling (Wardlaw et al. 1989). Using this factor (3–4 % loss per 1 °C above 15–20 °C), it can be calculated that most commercially sown wheat cultivars in India would lose approximately 50 % of their yield potential when exposed to 32–38 °C temperature at the crucial grain formation stage. It has been observed that a heat wave (35–37 °C) of 3–4 days modifies grain morphology and reduces grain size (Wardlaw and Wrigley 1994). For example, a short period (4 days) of exposure to high temperature (>35 °C) reduced grain yield up to 23 % (Stone and Nicolas 1994) and 3-day heat treatment (~38 °C from 8 am to 5 pm) reduced individual yield component up to 28.3 % (Mason et al. 2010).

According to Hays et al. (2007), 32–35 °C is common in the Southern Great Plains of the US where roughly 30–40 % of the country's wheat crop is grown and in the northern region of Israel where more than 70 % of its wheat production is located. Furthermore, current estimates indicate that in India alone, more than 13.5 million ha of wheat growing area is heat stressed (Joshi et al. 2007c). Both the proximity to the equator and the popular rice–wheat cropping system, which involve late sowing of wheat, are the major causes of exposure of wheat in India and other neighboring countries to high temperatures during grain filling (Rane et al. 2007). The current trends in India indicate that the 'cool period' for wheat crop is shrinking, while the threat of terminal heat stress is increasing (Rane et al. 2007; Joshi et al. 2007b). Therefore, breeding for high-temperature tolerance in wheat is a major objective around the world. This need is expected to grow further in light of increased global warming (Lillemo et al. 2005). Hence, it is important to incorporate late heat tolerance into wheat germplasm. Breeding for heat tolerance is still in its infancy stage and warrants more attention in future (Ortiz et al. 2008; Ashraf 2010). Although, significant variation for heat tolerance exists among wheat germplasm (Reynolds et al. 1994; Joshi et al. 2007b, c), no direct criteria are available to do selection for heat tolerance. Phenotypic selection for heat tolerance has been

performed using indirect selection for GFD (Yang et al. 2002), thousand grain weight (TGW) and canopy temperature depression (CTD) (Reynolds et al. 1994; Ayeneh et al. 2002).

Heat tolerance is quantitative in nature and is controlled by a number of genes/quantitative trait loci (QTL) (Blum 1988), which may sometimes be involved in interactions with each other (QTL × QTL interaction) and/or with the environment (E) (QTL × E and QTL × QTL × E interactions). QTL for heat tolerance in wheat were reported using different traits like GFD, CTD and yield (Yang et al. 2002; Mason et al. 2010; Pinto et al. 2010) and senescence-related traits (Vijayalakshmi et al. 2010). Efforts are now being made to identify QTL using other important traits, such as yield, TGW and CTD. There are very few reports involving QTL analysis for the heat susceptibility index (HSI) of yield, TGW and GFD as well as CTD (under heat-stressed conditions) using a mapping population specially developed for heat tolerance and evaluated under natural field conditions of heat stress. However, HSI has been used effectively for measuring flooding tolerance in soybean (Githiri et al. 2006) and drought and heat tolerance in wheat (Kirigwi et al. 2007; Mohammadi et al. 2008; Mason et al. 2010).

During the past two decades, the use of marker-assisted selection (MAS) approaches has contributed greatly to a better understanding of the genetic basis of plant stress tolerance in some crops, in particular tomato and maize (Liu et al. 2006; Momcilovic and Ristic 2007) and, in some cases, has led to the development of plants with enhanced tolerance to abiotic stress (Lopes and Reynolds 2010; Thomson et al. 2010). Because of the general complexity of abiotic stress tolerance and the difficulty in phenotypic selection, MAS is considered an effective approach to improve this kind of tolerance. However, comparatively limited efforts have been made to identify genetic markers associated with heat tolerance in different plant species including wheat. Thus, there is an urgent need to understand the genetic factors affecting heat tolerance and to identify new diagnostic markers to be deployed in MAS breeding leading to improvement in grain yield of wheat under heat-stressed environments. Therefore, the present investigation was conducted with the objective of identifying markers associated with QTLs for terminal heat tolerance in hexaploid wheat.

## Materials and methods

### Plant material

A mapping population consisting of 148 RILs developed by making crosses between two parental genotypes,

NW1014 (heat tolerant) and HUW468 (heat susceptible) was used in the present study. NW1014 is recommended for planting under late-sown conditions of the North Eastern Plain Zone (NEPZ) of India while HUW468 is recommended for cultivation under timely sown conditions of NEPZ (Joshi et al. 2007b) and does not perform well under late (heat stressed) sowing conditions. Daily temperatures for the duration of early and late-sown experiments for 2 years (Fig. 1) established that the late-sown experiment was under considerable heat stress compared to the normal sown.

#### Development and evaluation of RILs for heat tolerance

Development and evaluation of the RILs was carried out at the research farm of the Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India, which is located in the NEPZ of India. Wheat grown in the NEPZ is exposed to high temperatures (Joshi et al. 2007c) and is considered a heat-prone mega-environment (ME), classified as ME5 by the CIMMYT (Braun et al. 1992). An off-season facility at the Regional Research Station, Indian Agricultural Research Institute (IARI), Wellington, Tamil Nadu, India, was used for advancing the generations.

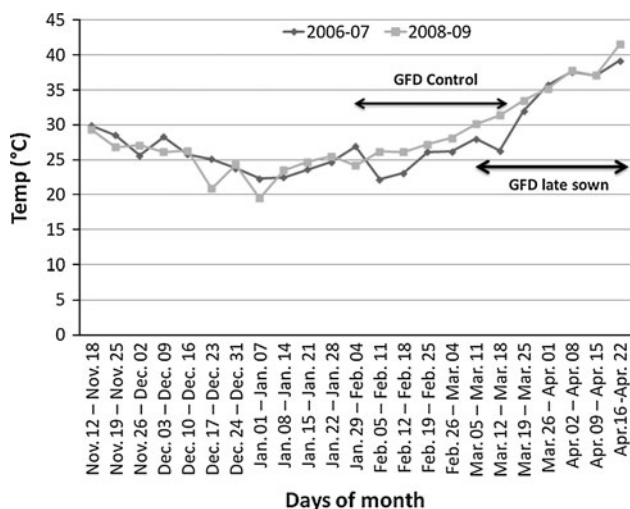
Development of RILs was achieved following the method described by (Singh and Rajaram 1991; Joshi et al. 2004). The cross was made in the year 2002–2003 and the  $F_1$  was grown in the off-season nursery (2003). The  $F_2$  was grown as space sown in 2003–2004 main season and the  $F_3$  generation was obtained by harvesting random  $F_2$  plants. The  $F_4$ ,  $F_5$ , and  $F_6$  generations were derived in the same way by harvesting one random plant from each progeny row in each generation. However, the requirement

of seed quantity for a proper replicated trial in  $F_5$ ,  $F_6$ , and  $F_7$  generations (as done in this study) was larger than that could be possible by growing seeds of a single plant. Therefore, once  $F_5$ ,  $F_6$ , and  $F_7$  RILs were obtained, each of the progeny rows was bulked separately to grow replicated trials in the next crop cycle. In other words, RILs were developed using a single random plant in each generation, while bulking was done in three generations ( $F_5$ ,  $F_6$ , and  $F_7$ ) to obtain sufficient quantity of seeds to plant replicated trials. Since all the plants of a progeny row were bulked in a generation, the experimental plot grown in the next cycle represented the same previous generation.

This way, RILs were evaluated in  $F_5$ ,  $F_6$ , and  $F_7$  generations. Observations on  $F_5$  and  $F_6$  generations were recorded in 2006–2007, while for  $F_7$  generation this was done in 2008–2009. Each generation ( $F_5$ ,  $F_6$ , and  $F_7$ ) was considered a trial. A line showing lower values of HSI and % decline but higher CTD was considered tolerant.

The  $F_5$  and  $F_6$  generations (148 lines) was planted at two different sowing dates (normal = third week of November 2006 considered as control; very late sown = first week of January 2007 considered as heat stressed) in three replications in 2006–2007. Each replication consisted of four blocks having 39 lines with a check (HUW 468) after every fifth line to serve as covariate in ANOVA analysis. The covariate value for each line was 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 were used. Each RIL was hand sown using a randomized complete block design in four-row plots of 3 m<sup>2</sup> area with 25 cm spacing between the rows. The two dates of sowing were planted such that genotype 1 in replicate 1 of the normal trial was also the genotype 1 in the replicate 1 of late sown. All other genotypes followed the same planting approach. The  $F_7$  generation was grown in the same manner in 2008–2009. The delayed planting (very late sown) allowed post-anthesis stages to coincide with warmer temperatures during the month of March and early April. The average temperatures between anthesis to physiological maturity in normal sown conditions were 25.6 and 27.4 °C in the 2 years, while in late sown, the temperatures were 34.6 and 36.3 °C, respectively (Fig. 1).

Agronomic practices recommended for irrigated and normal fertility (120 kg N; 60 kg P<sub>2</sub>O<sub>5</sub> and 40 kg K<sub>2</sub>O ha<sup>-1</sup>) conditions were followed throughout the 2 years. Full doses of K<sub>2</sub>O and P<sub>2</sub>O<sub>5</sub> were applied at the time of sowing. Nitrogen was supplied in the form of split application, 60 kg N ha<sup>-1</sup> at sowing, 30 kg N ha<sup>-1</sup> at first irrigation (21 days after sowing) and 30 kg N ha<sup>-1</sup> at second irrigation (45 days after sowing). A total of five



**Fig. 1** Daily mean temperatures of wheat growing seasons (2006–2007 and 2008–2009)

irrigations were given in each experiment. For proper evaluation of heat stress, the fungicide Tilt (propiconazole; [1-([2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl)-1H-1,2,4-triazole]) was applied (625 g a.i./ha) at two growth stages (GS), GS54 and GS69 (Zadoks et al. 1974) to prevent spot blotch and leaf rust, the two most important diseases of wheat in eastern India.

#### Variables measured

Data were recorded for grain yield ( $\text{kg ha}^{-1}$ ), TGW (g), date of anthesis (anthers extruding from 75 % of the plants per RIL plot) and days to maturity (complete loss of green color from the glumes of 75 % of the plants per RIL plot) for all RILs and parental genotypes. The GFD was calculated using the interval between the date of anthesis and physiological maturity. Canopy temperature ( $^{\circ}\text{C}$ ) was recorded in the late-sown crop. Based on the TGW data of two dates of sowings, HSITGW was calculated using the following formula (Fischer and Maurer 1978):

$$\text{HSITGW} = [(1 - \text{TGW}_{\text{heatstress}}/\text{TGW}_{\text{control}})/D]$$

where,  $\text{TGW}_{\text{heat stress}} = \text{TGW}$  in very late sowing conditions;  $\text{TGW}_{\text{control}} = \text{TGW}$  in normal sowing conditions

$$D(\text{stress intensity}) = (1 - X_{\text{heatstress}}/X_{\text{control}})$$

where,  $X_{\text{heat stress}} = \text{mean of TGW}_{\text{heat stress}}$  of all RILs;  $X_{\text{control}} = \text{mean of TGW}_{\text{control}}$  of all RILs. Similarly, HSI of GFD (HSIGFD) was calculated from the data recorded on the respective dates of sowing.

Canopy temperature was recorded on each plot (4 rows) using a handheld infrared thermometer (IMPAC Electronic GmbH, Germany) on bright sunny days between 1 and 3 pm at 7-day intervals, starting from 7 days after anthesis. Three recordings were made. For each plot, measurements were made at approximately 0.5–1 m distance from the edge of the plot and approximately 50 cm above the canopy with an approximate angle of  $30^{\circ}$ – $60^{\circ}$  from horizontal giving a canopy view of  $10 \text{ cm} \times 25 \text{ cm}$  (Ayeneh et al. 2002). Ambient temperatures were measured, after recording observations in each plot, using a handheld thermometer. A nonsignificant minor ( $\pm 0.5^{\circ}\text{C}$ ) change was observed in the ambient temperature during individual recordings date. The ambient temperatures during first recording in F5, F6 and F7 were 31.5, 32 and 31  $^{\circ}\text{C}$ , respectively, while for the second date recordings were 35.5, 35.5 and 34  $^{\circ}\text{C}$ . Although, we recorded CT three times but the data of the third date was not used since the lines were senescing and not much variation was visible among the lines. CTD was calculated using the following formula:

$$\text{CTD} = \text{Ambient temperature} - \text{canopy temperature.}$$

#### DNA isolation and simple sequence repeat (SSR) analysis

Genomic DNA was isolated from 15-day-old seedlings using the modified CTAB method described by Doyle and Doyle (1990). DNA was diluted in distilled  $\text{H}_2\text{O}$  to a concentration of  $5\text{--}10 \text{ ng } \mu\text{l}^{-1}$  before use in polymerase chain reaction (PCR).

PCR with SSR markers (*gwm*, *wmc*, *swm*, *barc* and *cfid*) were performed as described by Röder et al. (1998) and Somers et al. (2004). DNA amplification and fragment detection were done as described by Kumar et al. (2009). The annealing temperature of SSR primers was determined according to the information provided with the primers (Röder et al. 1998; Somers et al. 2004). Fragment sizes of amplified products were calculated using computer program 'Fragment Analyzer Version 1.02' by comparison with the internal and external size standards as described by Kumar et al. (2009).

#### Map construction and QTL detection

For the preparation of a whole genome framework molecular map, a set of 560 SSR primer pairs (*gwm*, *swm*, *wmc*, *barc*, and *cfid*), located on all 21 wheat chromosomes were selected from the reference International Triticeae Mapping Initiative (ITMI) maps (Röder et al. 1998; Ganal and Röder 2007). Approximately 25 SSRs evenly placed on each chromosome were selected to cover the whole genome. The parents were screened with the selected primers and once polymorphism was identified between the parents, 74 extreme RILs (37 most tolerant and 37 most susceptible) were genotyped with the polymorphic primers. Using 74 extreme lines and polymorphic primers, a framework linkage map was prepared with Mapmaker v2.0 (Lander et al. 1987) and preliminary QTL analysis was performed using QTL Cartographer v2.5 (Wang et al. 2005). Afterwards, all chromosomal regions found to be significant in preliminary analysis were enriched with more microsatellite markers and all available RILs (148) were genotyped with all polymorphic markers. For QTL identification as well as QTL  $\times$  QTL analysis, complete genotypic data set was used since all the markers of QTL regions were deployed to genotype all the RILs. The 74 RILs were used only in preliminary analysis. Later the analysis was performed again with all the RILs and all the markers from QTL regions for cross-validation. The linkage map was constructed using a likelihood of odds ratio (LOD) of  $>3$  and recombination fraction of  $<0.4$ . The commands 'Order' and 'Rip' were used to assign the order of markers to the map. The 'Try' and 'Compare' commands were used to include additional markers on the map.

Composite interval mapping (CIM) was performed using QTL Cartographer v2.5. The trait settings for CIM were model 6, forward and backward stepwise regression with a threshold of  $P < 0.05$  to select cofactors, window size 10 and 2 cM walking speed along chromosomes. QTL were verified by LOD scores compared to an empirical genome-wide significant threshold calculated from 1,000 permutations for  $P < 0.01$ . LOD scores and coefficients of determination were estimated by CIM for each QTL. We also accepted those QTL as significant at LOD value 2 and more if QTLs were observed in more than one environment. McCartney et al. (2005) and Pinto et al. (2010) and reported LOD threshold between 2 and more for accepting a QTL as significant if QTL fulfilled all the declaration criteria and was mapped in the same region as QTL controlling other traits. For ease of understanding, LOD line was placed at 2.0. Mean data for all three traits (HSITGW, HSGFD and CTD) in each generation ( $F_5$ ,  $F_6$ , and  $F_7$ ) and data pooled over generations were used for QTL analysis. Test of homogeneity was performed before pooling the data from the three different trials. The names of the QTLs detected in present study were designated according to the International Rules of Genetic Nomenclature (<http://wheat.pw.usda.gov/ggpages/wgc/98/Intro.htm>), consisting of a 'Q' followed by ht (heat tolerance), institution designation, and chromosome assignment with QTL position on same chromosome. QTL for HSITGW, HSGFD and CTD (very late sown) traits that co-localized within the same genomic region were assigned a common QTL name.

### Statistical analysis

Analysis of variance (ANOVA) for HSITGW, HSGFD, and CTD as well as phenotypic correlation coefficient between the traits for three trials was performed using GenStat-12.1/2009 (<http://www.vsni.co.uk/genstat>) program. Heritability of each trait in the population was estimated using following formula (Nyquist 1991):

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \left(\frac{\sigma_{gt}^2}{t}\right) + (\sigma^2/rt)}$$

where,  $h^2$  represents the heritability,  $\sigma_g^2$  is genetic variance,  $\sigma_{gt}^2$  is genotype  $\times$  trial variance,  $\sigma^2$  is error variance,  $r$  is number of replications and  $t$  is the number of trials.

## Results

### Phenotypic assessment of RILs

Mean and range of agronomic and physiological traits for parents and RILs in all three trials are presented in Table 1.

Mean values of HSITGW, HSGFD, and HSIYLD for the heat-tolerant parent (NW1014) ranged from 0.46 to 0.54, 0.68–0.83, and 0.60–0.69, respectively. On the other hand, the values of HSITGW, HSGFD, and HSIYLD for the susceptible parent (HUW468) ranged from 1.40 to 1.70, 1.42–1.48, and 1.21–1.30 (Table 1). Furthermore, the mean CTD values (5.68–6.76) of the tolerant parent was significantly higher than the susceptible parent (2.89–3.81) across the all the three trials.

The total growth period (crop emergence to physiological maturity) of RILs under normal and late-sown conditions averaged 122 days (trial 1) and 100 days (trial 3), respectively. This showed, on an average, 22 days (18 %) reduction in crop period due to late sowing. Likewise, the mean GFD of RILs under normal and late-sown conditions was 34 days (trial 1) and 25 days (trial 2), respectively (Table 1) showing a reduction of 9 days (25.7 %) due to late sowing. The mean TGW of RILs under normal and late sown was 37.5 g (trial 2) and 28.9 g (trial 2), respectively, which showed 8.6 g (23.1 %) reduction of RILs under late-sown condition as compared to control. The number of heads per  $m^2$  in normal sown trials was in the range of 350–400 while for late sown it was around 250–300. The mean yield of RI lines under normal (1.14 kg per plot) (trial 1) and late sown (0.69 kg per plot) (trial 2) accounted for 0.45 kg (39.4 %) reduction due to late sowing. The yield obtained is comparable to that of farmers fields in the region.

The HSI of TGW and YLD ranged from 0.27 to 1.93 and 0.26–1.68, respectively, across the trials (Table 1).

The RILs showed continuous distribution for all traits (HSITGW, HSGFD, HSIYLD, and CTD) of heat tolerance (Fig. 2a–d). The Shapiro–Wilk test performed on the mean values of HSITGW ( $W = 0.9885$ ,  $P$  value = 0.2554), HSGFD ( $W = 0.9935$ ,  $P$  value = 0.7393), HSIYLD ( $W = 0.9761$ ,  $P$  value = 0.3826) and CTD ( $W = 0.9937$ ,  $P$  value = 0.7601) revealed that the RILs fitted a normal distribution. Transgressive segregants were also observed among the RILs.

Analysis of variance for all four traits under normal and late-sown conditions and HSI of TGW, yield, and GFD traits showed significant variation for RILs and RILs by trial interaction (Table 2). Similarly, ANOVA for CTD revealed significant variation for RILs and RILs  $\times$  trial interaction (Table 2). The broad sense heritability for all measured traits in control and late sown and HSI of TGW, yield and GFD ranged from 35 to 89 % (Table 2).

### Correlation between traits of heat tolerance

The correlation between traits ranged from 0.01 to 0.78. The traits GFD, TGW, and CTD showed significant correlation with one another under late-sown condition including that

**Table 1** Mean values of parental genotypes and recombinant inbred lines (RILs) and range values of RILs for measured traits including HSITGW, HSIYLD and HSIGFD in control (C) and late sown (LS) (heat stress) conditions of three trials in 2 years (2006–2007 and 2008–2009) of testing in India

Traits	NW1014		HUW468		RILs mean		RILs range	
	C	LS	C	LS	C	LS	C	LS
Trial 1 (2006–2007)								
DA (days)	91.14 ± 1.11	78.33 ± 1.52	88.21 ± 1.48	80.66 ± 1.92	87.00 ± 4.15	77.22 ± 2.0	78–99	71–85
DM (days)	127.36 ± 1.32	105.66 ± 2.08	125.86 ± 1.18	103.18 ± 1.73	122.21 ± 3.00	103.00 ± 2.1	116–134	98–111
GFD (days)	36.14 ± 1.02	27.35 ± 0.98	37.45 ± 1.12	22.87 ± 1.37	34.30 ± 2.10	25.50 ± 1.60	29–40	21–30
TGW (g)	44.51 ± 0.56	37.48 ± 1.04	40.76 ± 0.98	25.31 ± 1.07	45.19 ± 4.20	33.80 ± 4.50	34.9–49.4	23.00–38.11
YLD (kg/plot)	1.58 ± 0.10	1.16 ± 0.09	1.72 ± 0.12	0.87 ± 0.06	1.14 ± 0.08	0.70 ± 0.04	0.84–1.73	0.43–1.16
CTD (°C)	5.68 ± 0.42		2.89 ± 0.6		4.89 ± 0.62		2.39–7.28	
HSITGW	0.54 ± 0.06		1.40 ± 0.04		0.95 ± 0.18		0.34–1.73	
HSIGFD	0.83 ± 0.05		1.48 ± 0.03		0.84 ± 0.12		0.29–1.30	
HSIYLD	0.69 ± 0.07		1.28 ± 0.03		0.98 ± 0.11		0.31–1.62	
Trial 2 (2006–2007)								
DA (days)	91.33 ± 1.85	78.43 ± 1.15	89.33 ± 2.02	76.31 ± 1.32	90.43 ± 4.05	80.21 ± 3.10	82–105	74–89
DM(days)	126.6 ± 1.20	106.50 ± 1.17	128.32 ± 1.85	99.66 ± 1.33	125.21 ± 3.14	106.20 ± 2.43	118–139	100–110
GFD (days)	35.72 ± 1.15	27.86 ± 1.08	39.12 ± 1.04	23.42 ± 1.53	35.20 ± 2.1	26.10 ± 1.9	30–41	22–31
TGW (g)	43.76 ± 1.16	37.48 ± 1.08	46.28 ± 1.10	27.2 ± 1.01	37.50 ± 4.30	28.90 ± 4.8	26.31–47.85	19.72–37.96
YLD (kg/plot)	1.55 ± 1.58	1.13 ± 1.15	1.74 ± 1.55	0.85 ± 1.21	1.21 ± 5.72	0.69 ± 5.21	0.67–1.71	0.45–1.13
CTD (°C)	6.76 ± 0.33		3.81 ± 0.21		5.90 ± 0.47		2.92–6.45	
HSITGW	0.46 ± 0.02		1.49 ± 0.05		1.11 ± 0.09		0.30–1.81	
HSIGFD	0.76 ± 0.07		1.45 ± 0.05		0.99 ± 0.15		0.40–1.76	
HSIYLD	0.64 ± 0.03		1.21 ± 0.05		0.99 ± 0.08		0.26–1.68	
Trial 3 (2008–2009)								
DA (days)	86.66 ± 2.33	75.10 ± 2.18	87.35 ± 2.84	73.21 ± 2.30	88.12 ± 2.67	74.6 ± 2.20	78–96	68–80
DM(days)	122.30 ± 2.46	101.67 ± 1.52	124.60 ± 2.66	96.80 ± 1.73	123.22 ± 2.10	100.14 ± 2.3	113–129	96–105
GFD (days)	34.28 ± 1.34	26.66 ± 1.47	33.69 ± 1.19	22.78 ± 1.21	34.72 ± 2.03	25.82 ± 1.80	30–41	23–30
TGW (g)	44.17 ± 1.23	37.83 ± 0.83	45.05 ± 1.45	24.79 ± 1.13	42.61 ± 3.72	30.83 ± 3.44	33.34–49.22	20.11–39.10
YLD (kg/plot)	1.56 ± 2.21	1.14 ± 1.92	1.75 ± 2.14	0.85 ± 2.26	1.23 ± 5.37	0.74 ± 5.60	0.84–1.75	0.46–1.14
CTD (°C)	6.16 ± 0.53		3.00 ± 0.26		4.81 ± 0.33		2.5–7.17	
HSITGW	0.48 ± 0.08		1.70 ± 0.12		1.03 ± 0.13		0.27–1.93	
HSIGFD	0.68 ± 0.04		1.42 ± 0.09		1.02 ± 0.10		0.31–1.68	
HSIYLD	0.60 ± 0.04		1.30 ± 0.05		0.99 ± 0.14		0.42–1.52	

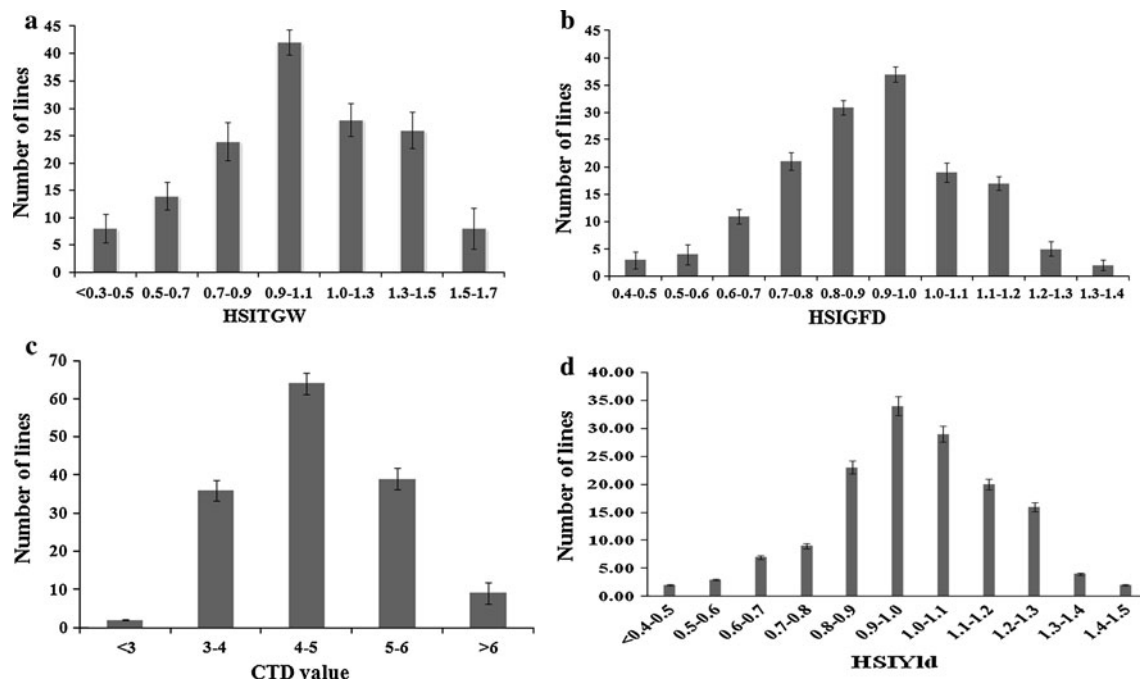
± indicates standard error

with HSITGW, HSIYLD, and HSIGFD (Table 3). Similarly, yield also had significant correlation with other traits (except maturity) in late-sown condition and also with HSITGW, HSIYLD, and HSIGFD (Table 3). The HSIGFD had significant negative correlation with all measured traits except days to anthesis (DA) in the late-sown trials, while in control conditions it showed significant correlation with DA and YLD. Likewise, HSITGW displayed significant correlations in late-sown conditions with all measured traits except DA, while HSIYLD showed significant correlations with DA, GFD, TGW, YLD, CTD, and HSITGW. Days to maturity (DM) was significantly correlated with DA, TGW,

GFD, HSITGW, and HSIGFD under control and late-sown conditions.

#### SSRs polymorphism and linkage map constructions

Five hundred and sixty (560) SSR primer pairs representing all 21 chromosomes of wheat were used to detect polymorphism between the parental genotypes, NW1014 (heat tolerant) and HUW468 (heat susceptible). Out of the 560 SSR primer pairs, polymorphism between the parents was detected by 182 (32.5 %) SSRs. Of these 182 SSR markers, 22 markers were unlinked and were not used for linkage



**Fig. 2 a–d** Distribution of heat susceptibility index of thousand grain weight (HSITGW), heat susceptibility index of grain filling duration (HSIGFD), canopy temperature depression (CTD) and heat susceptibility index of YLD (HSIYLD) values averaged over 2 years and

three trials for 148 recombinant inbred lines (RILs) derived from the cross ‘NW1014’ × ‘HUW468’. Bars standard errors; however, some error bars are smaller than the symbol and may not be visible

map construction. Thus, 160 SSR were used to construct 21 linkage maps of NW1014 × HUW468 population. The linkage maps spanned 3,698.8 cM with an average distance of 23.11 cM per marker (Supplementary Fig. 1). The maximum number of markers was mapped on the A genome (36.25 %) followed by the B (34.38 %) and D (29.37 %) genomes. Markers were unevenly distributed between linkage groups. The number of mapped loci per linkage group ranged from 3 (chromosomes 4A, B, D) to 16 (chromosome 2A). The positions of centromeres and all marker loci on the linkage groups of all 21 chromosomes were assigned using the information from the deletion map developed by Sourdille et al. (2004) and a recent genetic map of the ITMI population (Ganal and Röder 2007).

#### QTL analysis

Results of QTL analysis are shown for four different traits of heat tolerance (Fig. 3a–h). Using CIM, three genomic regions for heat tolerance were detected on chromosomes 2B, 7B and 7D in all three trials (Fig. 3a–h). The LOD values of identified QTL ranged from 2.0 (*Qls-dum.bhu-7D*) to 9.7 (*QHthsitgw.bhu-7B*) explaining 7.42 and 25.39 % phenotypic variation, respectively (Table 4).

The QTL identified on the long arms of chromosome 2B was flanked by the markers *Xgwm935–Xgwm1273*

(23 cM). The alleles for enhanced heat tolerance of this QTL were derived from the heat-tolerant parent NW1014 for the traits HSITGW and HSIGFD. The QTL appeared consistent and explained substantial phenotypic variation for HSITGW (17.82 %) and HSIGFD (20.29 %) over mean of the three trials. For HSIGFD, it explained phenotypic variation as high as 29.36 % in the second trial.

The QTL identified on the long arms of chromosome 7B was flanked by the markers *Xgwm1025–Xgwm745* (3.6 cM). Like the QTL on chromosome 2B, the alleles for enhanced heat tolerance for the QTL on 7BL were derived from the heat tolerant parent NW1014 for HSITGW and HSIYLD as well as CTD and YLD under late-sown conditions. Of all the QTLs, *QHthsitgw.bhu-7B* explained highest phenotypic variation (20.34 %) for HSITGW based on the mean values over three trials, while in the third trial it explained maximum phenotypic variation (25.39 %) for the trait. This was the QTL that explained significant phenotypic variation (19.81 %) for CTD across mean of trials with maximum of 20.29 % in the second trial.

The third QTL identified on the short arm of chromosome 7D was flanked by marker interval *Xgwm3062–Xgwm4335* (3.1 cM). Unlike the previous two QTLs, the allele for enhanced heat tolerance in chromosome 7DS for HSITGW and DM under late-sown conditions was

**Table 2** Analysis of variance (ANOVA) and heritability ( $h^2$ ) for measured traits in control and late sown including HSIYLD, HSIYLD and HSIYLD in very late sown of 148 recombinant inbred lines (RILs) derived from the cross NW1014 (heat tolerant) × HUW468 (heat susceptible) of wheat tested in three trials

Source of variation		Mean sum of squares														
	df	C_YLD	LSYLD	C_TGW	LS_TGW	C_GFD	LS_GFD	CTD	C_DA	LS_DA	C_DM	LS_DM	HSIYLD	HSITGW	HSIGFD	
Covariate	1	277.2**	17.3**	12,789.8**	121.2**	0.001	16.9**	0.001	137.2*	1,819.0**	240.6*	3,877.4**	0.001	0.001	0.066	
Trials	2	3,367.6**	605.3**	7,509.9*	2,688.9**	51.6*	22.4*	106.45**	552.9**	2,261.6**	854.1**	1,763.5**	0.013**	0.144**	0.326**	
Rep (trials)	6	12.9**	2.4**	1,138.2**	3.52	6.3**	4.9**	1.163**	36.04**	8.6*	40.5**	11.6**	0.012**	0.032	0.485**	
RILs	147	381.3**	271.7**	108.6**	148.4**	28.3**	20.4**	6.41**	72.6**	24.7**	40.5**	16.2**	0.346**	0.768**	0.459**	
Trials × RILs	294	130.3**	93.2**	19.5**	16.7**	5.6**	3.7**	1.24**	24.1**	12.2**	19.0**	10.5**	0.088**	0.110**	0.057*	
Residuals	881	1.4	0.7	5.56	2.92	0.88	0.816	0.04	7	3.93	5.9	3.15	0.001	0.033	0.025	
Heritability		0.66	0.66	0.82	0.89	0.80	0.82	0.81	0.67	0.51	0.53	0.86	0.740	0.86	0.87	

C control, LS late sown

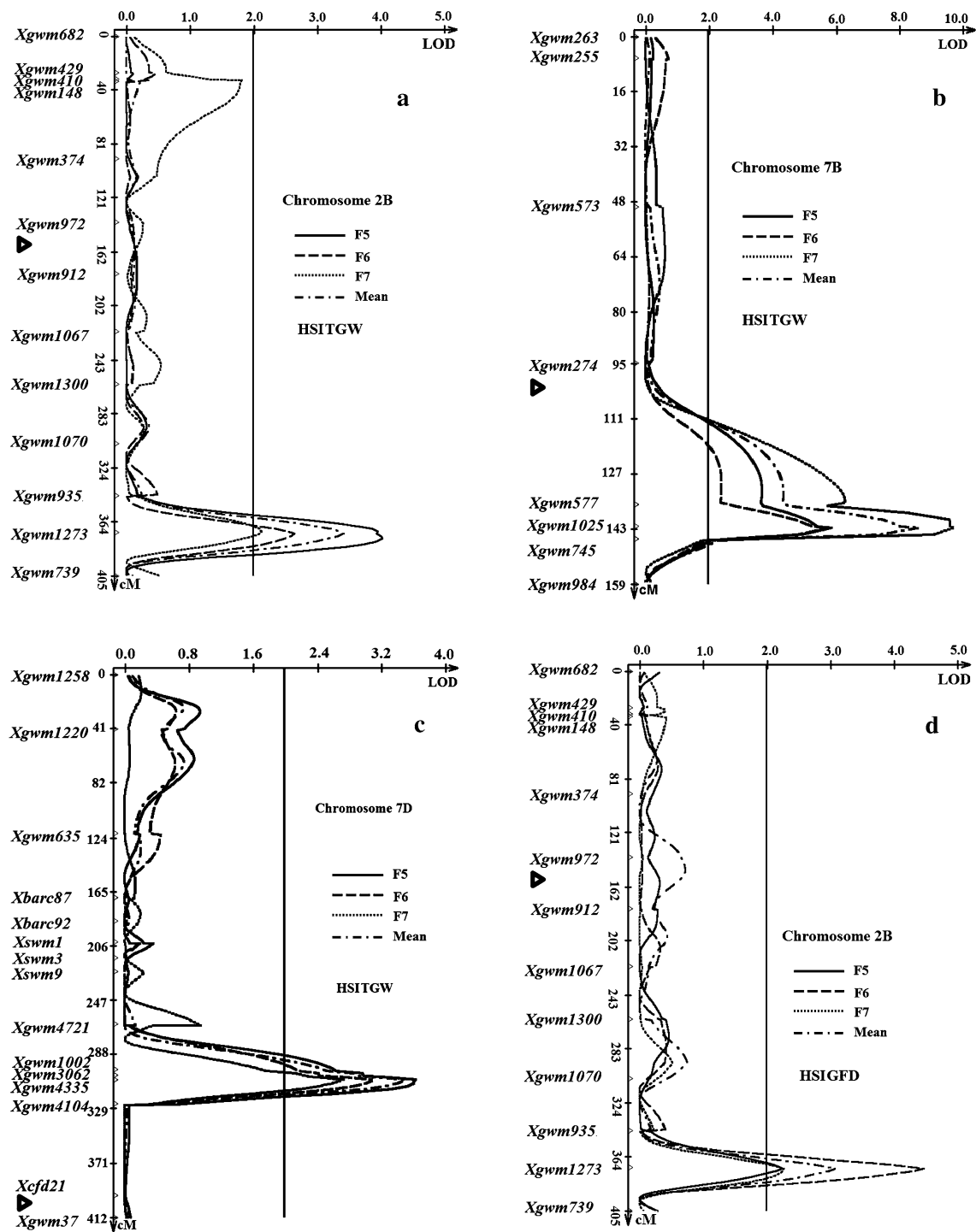
\* Significant at  $P = 0.05$ ; \*\* significant at  $P = 0.01$

**Table 3** Pearson's correlation coefficients of measured traits in control (C) and late sown (LS) conditions including HSIYLD, HSIYLD and HSIYLD in 148 recombinant inbred lines (RILs) of the cross NW1040 (heat tolerant) × HUW468 (heat susceptible) of wheat in across all three trials

Traits	C_DA	C_GFD	C_DM	C_TGW	C_YLD	LS_DA	LS_GFD	LS_DM	LS_TGW	LS_YLD	CTD	HSIGFD	HSITGW
C_DA	1												
C_GFD	-0.66**	1											
C_DM	0.78**	-0.05	1										
C_TGW	-0.32**	0.26**	-0.20*	1									
C_YLD	-0.04	0.11	0.09	0.28**	1								
LS_DA	0.39**	-0.24**	0.33**	-0.19*	-0.05	1							
LS_GFD	-0.09	0.21**	0.06	0.20**	0.22**	-0.64**	1						
LS_DM	0.38**	-0.06	0.46**	0.06	0.17*	0.50**	0.32**	1					
LS_TGW	-0.15*	0.22**	0.03	0.67**	0.25**	-0.25**	0.42**	0.18*	1				
LS_YLD	-0.07	0.26**	0.12	0.30**	0.44**	-0.23**	0.36**	0.11	0.53**	1			
CTD	-0.11	0.24**	0.06	0.43**	0.34**	-0.29**	0.42**	0.15*	0.75**	0.58**	1		
HSIGFD	-0.35**	0.42**	-0.12	-0.07	-0.15*	0.35**	-0.67**	-0.33**	-0.23**	-0.16*	-0.24**	1	
HSITGW	-0.12	-0.09	-0.24**	0.02	-0.10	0.11	-0.35**	-0.27**	-0.69**	-0.41**	-0.64**	0.26**	1
HSIYLD	0.06	-0.15*	-0.05	-0.12	0.29**	0.19*	-0.22**	0.01	-0.39**	-0.73**	-0.36**	0.07	0.37**

\* Significant at  $P = 0.05$ ; \*\* significant at  $P = 0.01$ ; without \* nonsignificant





**Fig. 3 a–h** Likelihood of odds ratio (LOD) curves obtained by composite interval mapping for quantitative trait loci (QTL) mapped on chromosomes 2B, 7B and 7D **a–c** for heat susceptibility index of thousand grain weight (HSITGW); **d** heat susceptibility index of grain filling duration (HSIGFD), **e** days to maturity and canopy temperature

depression (CTD); **f, g** late-sown yield and heat susceptibility index of YLD (HSIYLD); **h** days to maturity in the RIL mapping population of ‘NW1014’ × ‘HUW468’. Vertical lines the threshold LOD value (2.0) determining significant QTL. Short arms are on the top and open triangles indicate the probable position of centromeres

contributed by the heat-susceptible parent HUW468. This QTL also appeared consistent but explained lowest phenotypic variation (9.78 %) compared to the other two main

QTL for HSITGW based on mean values over the three trials. Its contribution to DM under late sown was also moderate (7.42 %) and lacked consistency.

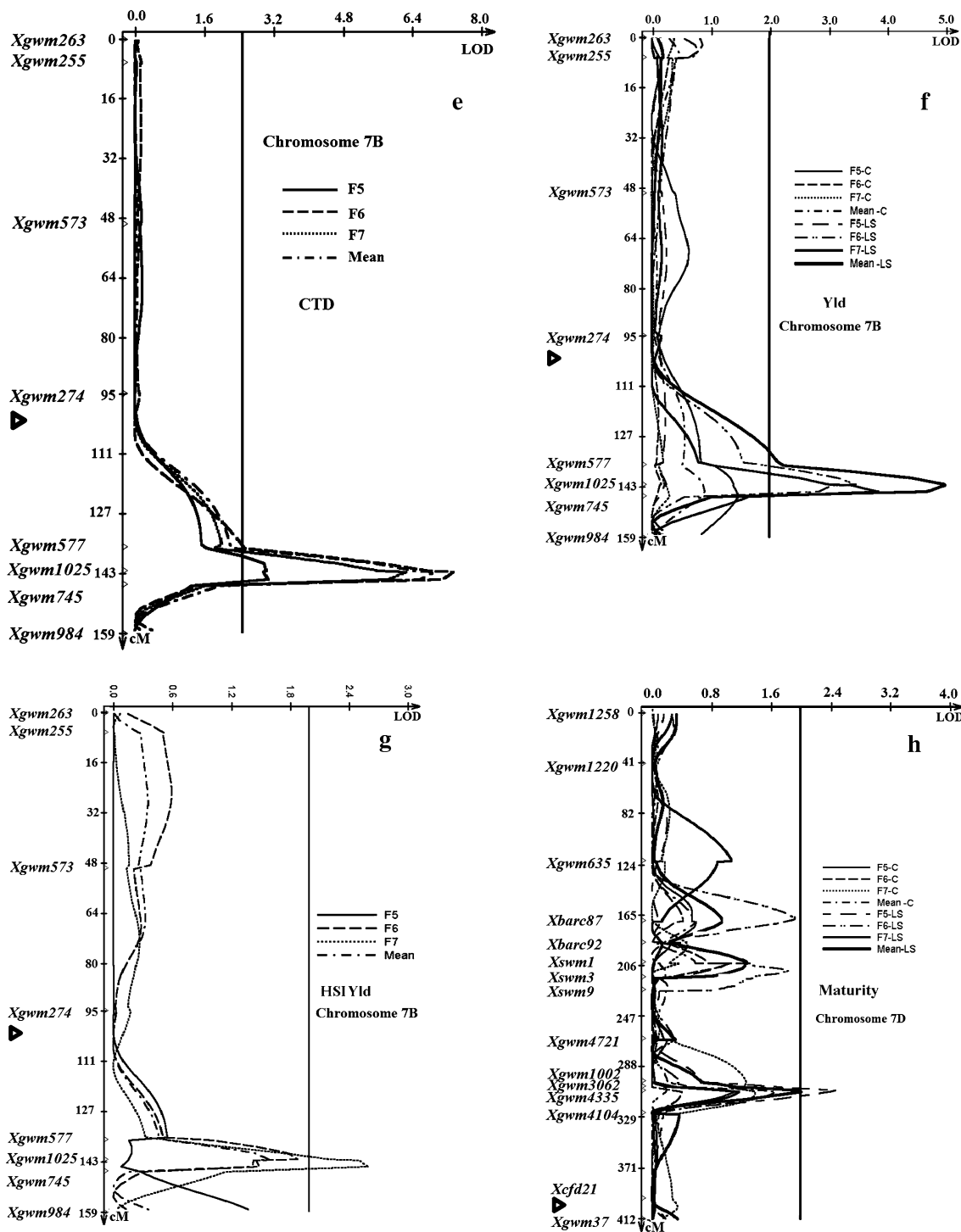


Fig. 3 continued

**Discussion**

Phenotypic analysis of RILs for QTL mapping

The distributions of the mean RIL values were normal across trials for HSITGW, HSIYLD, HSI GFD and CTD

(under very late-sown conditions) and indicated continuous variation for heat tolerance traits (Fig. 2a–d). This supported the hypothesis of the quantitative nature of heat tolerance (Blum 1988). Mean values of some RILs exceeded the parental values indicating the presence of transgressive segregants in the population. As observed by

**Table 4** Effects of quantitative trait loci (QTL) for heat stress tolerance in NW1014 (heat tolerant)  $\times$  HUW468 (heat susceptible) recombinant inbred lines (RILs) detected by composite interval mapping (CIM) using QTL Cartographer (Wang et al. 2005)

Trait/QTL	Marker interval	Interval (cM)	Chrom	First year			Second year			Mean over trials										
				Trial 1 (F <sub>5</sub> )			Trial 2 (F <sub>6</sub> )			Trial 3 (F <sub>7</sub> )										
				LOD	R <sup>2</sup>	Add	LOD	R <sup>2</sup>	Add	LOD	R <sup>2</sup>	Add	LOD	R <sup>2</sup>	Add					
TGW																				
<i>QHhstsgw.bhu-2B</i>	Xgwm935-Xgwm1273	23.0	2BL	4.0	20.19	-0.34	2.6	16.50	-0.31	2.1	8.42	-0.39	3.4	17.82	-0.37					
<i>QHhstsgw.bhu-7B</i>	Xgwm1025-Xgwm745	3.6	7BL	5.9	14.02	-0.29	5.7	13.36	-0.26	9.7	25.39	-0.40	8.7	20.34	-0.34					
<i>QHhstsgw.bhu-7D</i>	Xgwm3062-Xgwm4335	3.1	7DS	3.6	12.68	0.35	3.1	9.36	0.33	2.7	7.21	0.39	3.5	9.78	0.38					
YLD																				
<i>QHhstYLD.bhu-7B</i>	Xgwm1025-Xgwm745	3.6	7BL	-	-	-	-	-	-	2.6	8.05	-0.80	-	-	-					
<i>QlsYLD.bhu-7B</i>	Xgwm1025-Xgwm745	3.6	7BL	-	-	-	3.4	8.06	2.56	3.6	11.23	2.83	4.4	13.21	3.40					
GFD																				
<i>QHhstgfd.bhu-2B</i>	Xgwm935-Xgwm1273	23.0	2BL	2.2	15.46	-0.25	4.5	29.36	-0.39	2.3	22.36	-0.23	3.1	20.15	-0.32					
CTD																				
<i>QHhctd.bhu-7B</i>	Xgwm1025-Xgwm745	3.6	7BL	3.1	10.36	0.21	7.4	20.29	0.29	6.3	17.89	0.22	6.9	19.81	0.24					
DM																				
<i>Qls-dm.bhu-7D</i>	Xgwm3062-Xgwm4335	3.1	7DS	2.5	8.12	-0.52	-	-	-	-	-	-	2.0	7.42	-0.38					

R<sup>2</sup> total percentage of phenotypic variance explained by each QTL, *add* the additive effect; a positive value indicates the NW 1014 allele having a positive effect on the trait, and negative value indicates HUW 468 allele having positive effect on the trait. *Q*  $\times$  *T* QTL  $\times$  trial interaction, *ns* nonsignificant, *LOD* likelihood of odds ratio

Yang et al. (2002), this suggested that the parents contributed different genes for heat tolerance and that traits were not simply inherited.

We used HSI and performance of traits under late-sown heat-stressed conditions as reported by other workers for similar objective (Mohammadi et al. 2008; Pinto et al. 2010; Yang et al. 2010; Barakat et al. 2011; Mason et al. 2010, 2011). HSI is considered a better parameter to select heat-tolerant plants and has been used in previous studies for measuring heat tolerance in crops like soybean and wheat (Ayeneh et al. 2002; Githiri et al. 2006; Kirigwi et al. 2007; Mohammadi et al. 2008; Mason et al. 2010, 2011). The performance of the same traits in control conditions was used to check whether the detected QTL was only for heat tolerance or represented the particular phenotypic trait when heat stress was absent. The presence of significant variation between RILs for all measured traits suggests that the RILs segregated for these traits and have normal distribution which allowed the detection of QTL associated with heat tolerance. Planting RILs in two experiments at two different sowing dates (normal and very late sowing date) provided ample opportunity to see the difference for HSI of traits and also the performance of measured traits under normal and heat-stressed conditions. The tolerant parent displayed lower values for HSI while higher for CTD in very late-sown trials which indicated a better cooling capacity during grain filling under higher temperature as reported by Reynolds et al. (1994) and Ayeneh et al. (2002). The degree of cooling reflects the rate of evapotranspiration on the surface of the plant canopy (Ayeneh et al. 2002) and gains in yield owing to the positive effect of reduced canopy temperature (Reynolds et al. 2007). The reduction in GFD and TGW due to terminal heat stress was also reported by Yang et al. (2002). Therefore, use of HSI, HSIYLD, and CTD as four different parameters to differentiate heat-tolerant and heat-susceptible lines appeared justified. The approach of mapping each yield component as a separate HSI also allows for the identification of QTL affecting single traits that would collectively contribute to overall yield stability and heat tolerance (Mason et al. 2010).

In our experiment broad sense heritability for GFD (under normal and late sown and HSIYLD) were quite high (85–87 %) as reported in earlier studies (Fokar et al. 1998; Yang et al. 2002; Pinto et al. 2010). The heritability of yield and TGW under normal and late-sown condition including their HSI were in agreement with Pinto et al. (2010) who reported heritabilities in the range of 68–71, 83–90, 54–86 % for TGW and yield ( $\text{gM}^2$ ) under normal, heat stressed, and drought conditions, respectively. This is one of the reasons that yield traits and GFD are the preferable criteria to screen for tolerance against abiotic stresses including heat and drought (Sharma et al. 2008; Yang et al.

2002; Pinto et al. 2010). The heritability for CTD was relatively moderate (81 %). Heritability of CTD has not been thoroughly evaluated; preliminary data suggest moderate to high heritability for the trait (Reynolds et al. 2001). It also showed high genetic correlation with yield and high values of proportion of direct response to selection (Reynolds et al. 2001), indicating that the trait is heritable and therefore amenable to early generation selection.

Negative correlation of CTD with HSIYLD, and HSIYLD indicates that RILs with high cooling capacity display lower HSI of TGW, YLD, and GFD. Saint Pierre et al. (2010) reported significant negative phenotypic correlation ( $r = -0.34$  to  $-0.75$ ,  $P < 0.001$ ) between canopy temperature and grain yield under drought conditions in wheat. Ayeneh et al. (2002) reported that canopy temperature can be used as a tool in the selection of wheat targeted for tolerance to heat stress. Most of the measured traits under late-sown conditions were significantly correlated to one another. The findings appear to suggest that under late-sown environment, CTD along with TGW and yield may be used as indirect selection criteria for heat tolerance in wheat.

The performance of the respective traits in control conditions was used to verify whether detected QTL was specific for heat tolerance or represented the particular phenotypic trait when heat stress was absent. The parents used to develop the mapping population did not differ significantly for days to anthesis and plant height. Additionally, we already excluded very early and very late flowering lines from the RILs to minimize the effect of flowering time. A population with synchronized phenology ensures that all the genotypes are exposed to the same environmental conditions in each of the growth stages (Reynolds and Tuberosa 2008) and therefore enhances the probability of identifying genes of minor effects such as those controlling a complex trait like yield (Reynolds et al. 2009). In other words, such population will facilitate in avoiding confounding of environmental patterns (Olivera-Villegas et al. 2007; Pinto et al. 2010). In this study, QTL for photoperiod and plant height were not observed either in normal or late-sown condition except days to maturity in the first late-sown trial. Absence of QTLs for phenological traits indicated that there were no confounding effects between environment and phasic development.

#### QTL mapping for heat tolerance

Three significant genomic regions on 2B, 7B, and 7D were found to be associated with heat tolerance. The genetic length of marker intervals obtained for QTLs on these three chromosomes (2B, 7B, and 7D) were 23.0, 3.6, and 3.1 cM, respectively. Although the marker interval in chromosome 2B was relatively larger, the high LOD value and co-localization indicated the presence of a significant

QTL in that chromosome region. Out of eight QTLs for heat tolerance detected using different parameters, four were major QTL contributing more than 15 % for phenotypic variation in pooled trials) which were detected on chromosomes 2B (for HSITGW and HSI GFD) and 7B (HSITGW and CTD). Using single-marker analysis, Yang et al. (2002) found QTLs associated with markers *Xgwm11* (1B) and *Xgwm293* (5A) for heat tolerance in an F<sub>2</sub> population (166 plants) that contributed 23 % of phenotypic variation. Mason et al. (2010) reported five stable QTL for HSI of single grain weight (1A and 2A), grain weight (3B), and grain number (2B and 3B) contributing 11.1–22.6 % of phenotypic variation. However, their phenotypic assessment was performed on 65 lines of F<sub>5</sub> generation material in controlled conditions for a short period of heat stress (3 days), while our study was performed under natural field conditions.

Since traits like heat stress tolerance are affected by the developmental stage of plants, we used the data of traits like DA, DM, GFD, TGW, and grain yield for the control and late-sown experiments separately to identify whether the QTLs for respective traits were for heat stress tolerance or for the physiological and developmental traits. The QTL analysis of DA, GFD, and TGW under control and late-sown condition revealed that heat stress tolerance QTLs did not co-localize with earlier reported QTLs for physiological and developmental traits. Likewise, the absence of QTLs in non-heat stress condition indicated that presented QTLs are only for heat tolerance. One of the QTL identified during the present study on the short arm of chromosome 7D (*QHttgw.bhu-7D*) was localized in the same genomic region (with a genetic distance of 4 cM to the closest marker *Xgwm1002*) where an important QTL, *QTgw.ipk-7D*, explaining more than 84.7 % of phenotypic variation for TGW was identified (Röder et al. 2008). Interestingly, the most potent QTL on chromosome 7BL is very close to the strong yield QTL *Xwmc273.2* on 7BL (map alignment from Ganai and Röder 2007) which was reported by Quarrie et al. (2005). The QTL region of 7B chromosome is also very close (6.4 cM) to another reported major QTL with linked marker *Xgwm577* which is a neighboring marker to the here reported QTL region of chromosome 7B and contributed 25 % phenotypic variation of heat tolerance using the parameter grain filling rate (Barakat et al. 2011). The QTL for stem water-soluble carbohydrates at the flowering stage and accumulation efficiency of stem water-soluble carbohydrates was also detected (Yang et al. 2007a, b) in the neighboring region of the QTL on chromosome 7BL. Pinto et al. (2010) reported important chromosomal regions of QTLs on chromosomes 1B, 2B, 3B, 4A, 4A, and 5A which were associated with 10, 9, 8, 7, 7, and 7 traits, respectively, for temperate irrigated, drought, and heat stress environments. Groos et al. (2003) suggested that QTL for TGW

may be used to improve yield due to its accurate detection and repeatability across environments in comparison to the QTL for yield.

In present study, QTL of HSITGW, HSI GFD, and CTD in the late-sown condition showed consistency in all the three trials. The QTLs located on chromosomes 2B and 7B for enhanced heat tolerance were derived from the heat-tolerant parent NW1014, while the susceptible parent, HUW468, contributed the allele for increased heat tolerance for HSITGW on chromosome 7DS. Mason et al. (2010) also detected QTLs which were contributed by the heat-susceptible cultivar for HSI of kernel number and kernel weight under heat stress.

Several co-localized QTL for yield and grain weight with agronomic and physiological traits have been reported in wheat under drought and heat stress and temperate irrigated conditions (Pinto et al. 2010; Mason et al. 2010). In this study, all three genomic regions for detected QTLs were collocated for HSITGW, HSI GFD, CTD, HSI YLD, LS-YLD, and LS-DM. The clustering of QTL to just a few genomic regions, as well as the coincidence of QTL locations and their maximum LOD scores within those regions increases the confidence that the QTLs obtained were real (Landjeva et al. 2010). These hot spot regions of QTL could carry the genetic information about relation between morpho-physiological and yield-related traits under heat-stressed environments. The presence of pleiotropic/co-localized QTLs and their importance has been reported in wheat for different traits (Kumar et al. 2010; Vijayalakshmi et al. 2010) including heat-stress-related traits (Mason et al. 2010, 2011; Pinto et al. 2010). According to Yang et al. (2007a, b), the common QTL can be a result of: (a) two strongly linked genes affecting different traits; (b) a single gene that can produce a number of effects in related traits; (c) one gene governing two or more independent traits; and (d) two linked genes that affect the same traits. This may be possible as Reynolds et al. (2007) reported that theoretical yield gains were associated with CTD under diverse environments. The co-location of QTL for different agronomic and physiological traits with QTL for yield suggests that it is possible to achieve genetic dissection of the crop performance under heat stress to facilitate a more efficient breeding approach (Pinto et al. 2010).

The novel and consistent QTLs obtained in this study that are associated with heat susceptibility index, late-sown performance of traits and canopy temperature depression could be exploited for molecular breeding by MAS (Yang et al. 2010) to improve heat tolerance in wheat. The observed QTLs probably resulted from allelic difference specific to the NW1014 × HUW468 cross, displaying new alleles that control heat tolerance and could be potentially exploited in future wheat breeding programs. There are no direct criteria which allow wheat breeders to select a

heat-tolerant line and hence they grow breeding populations and advanced lines under heat-stressed environment (late sown) for selection of heat-tolerant plants or lines. Furthermore, the traits like CTD, TGW, GFD, and yield are significantly affected by environment. Therefore, molecular markers identified in this study are expected to benefit wheat breeders in selection of heat-tolerant plants and will also provide preliminary information for further fine mapping and cloning. The QTL on chromosome 2B and 7B alone or together may be used in marker-assisted breeding to get enhanced level of heat tolerance. Although, the gap between markers on chromosome 2B is large, the use of both flanking markers rather than one is expected to give better accuracy.

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