Unravelling the genetic basis of terminal heat tolerance and yield related traits in bread wheat (*Triticum aestivum* L.)

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Abstract

Terminal heat stress is a critical abiotic stress in arid and semi-arid regions, causing substantial reductions in wheat productivity. Quantitative trait loci identification can aid marker-assisted selection in terminal heat tolerance wheat breeding by determining complex grain yield and its component traits. The present study reports the genetic dissection and QTL mapping for yield attributes and morpho-physiological traits using 200 recombinant inbred lines population, derived from WH711 (heat sensitive)/WH1021 (heat tolerant) grown under timely sown and late sown conditions during *rabi* 2018-19. Phenotyping was performed over 31 parameters, including morphological, physiological and yield attributes. Under heat stress conditions, a significant reduction was recorded in grain yield per plot and its component traits, *viz.* biological yield per plot, number of effective tillers per meter and gaseous parameters (photosynthetic rate, stomatal conductance and water use efficiency), whereas a reverse trend was observed in canopy temperature depression at anthesis and 15 days after anthesis stage based on total antioxidant activity and malondialdehyde content. Genetic diversity was assessed with the help of cluster analysis using 24 SSR markers data and 2-D and 3-D. PCA scaling broadly categorized the whole RIL population into two groups *i.e.,* heat tolerant and heat susceptible. Seven novel QTLs were identified through composite interval mapping on chromosomes 1B, 3B, and 7A, associated with two key yield attributes, two morphological traits, and a physiological trait under terminal heat stress conditions. The SSR marker Xwmc 596 was found to be linked with grain yield per plot, biological yield per plot and NDVI, that can be utilized as a good source of gene deployment and enhancement of heat tolerance in future breeding.

Keywords: RILs, diversity, bread wheat, heat tolerance, QTLs

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important cereal food crops of the world, widely adapted and holding first rank in cultivable areas globally (Maulana et al. 2018). The North-Western Plain Zone (NWPZ) of India contributes around 80% of total wheat production (Laxman et al. 2014). Due to short and mild winters in this zone, late reproductive phases of crop coincide with high temperatures which outturns detrimental effects on its grain filling phenomena (Rane et al. 2007). According to the estimates, a rise in temperature of each 1°C above the optimum temperature (15–20°C) is expected to reduce grain filling duration by 3.1 days and grain weight by 2.8 mg (Streck 2005), in addition to a drastic negative impact on grain filling duration, grain size, grain weight, harvest index and seed density. Heat stress also affects multiple physiological and gaseous phenomena like

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Table 1. List of parents used in the present study

S. No.	Genotype	Developing center	Pedigree	Characteristics
1	WH 711	CCSHAU, Hisar	ALD'S'HUAC/ HD2285/3/HFW-17	Dwarf variety with shining, amber, hard grains and good for chapati making. High yielding, lodging resistant and good quality grain. Haryana state for timely sown, high fertility and irrigated conditions. Moderately resistant to rust and Karnal bunt
2	WH 1021	CCSHAU, Hisar	NY0T95(GW 296)/ SONAK	Dwarf variety with shining, amber, hard grains. High protein content. Suitable for late sown, high fertility and irrigated conditions
3	200 RILs	CCSHAU, Hisar	WH711/WH1021	

chlorophyll content, photosynthetic rate, CO_2 assimilation and water-use efficiency (WUE) of wheat crop (Farooq et al. 2019; Zandalinas et al. 2018). High-temperature injury occurs primarily through photochemical reactions in thylakoid lamellae and carbon metabolism in chloroplast stroma (Wise et al. 2004; Wahid et al. 2007). Thereafter, it damages the plasma membrane, causing ionic leakage and production of reactive oxygen species and malondialdehyde content, which can be used to measure the severity of heat stress (Camejo et al. 2006; Guo et al. 2006). Afterward, these compounds enhance evapotranspiration and canopy temperature due to which the plant severely feels scarcity of water and performs very low WUE (Lamaoui et al. 2018). Therefore, chlorophyll content and relative amount of water (RWC) in leaf tissues play an imperative role in heat tolerance by maintaining greenness and gaseous exchange phenomena of leaves (Wahab et al. 2022). In response of oxidative stress, wheat plants make alterations in water relations, osmotic adjustment and production of antioxidant compounds in the tissues subjected to heat stress (Wahab et al. 2022; Kapoor et al. 2020).

Several factors, including morpho-physiological and gaseous exchange parameters, modulate the fate of heat tolerance in genotypes and hence, natural genetic variation in these parameters can be used as selection criteria to breed heat tolerant genotypes (Fathiand Tari 2016; Mitra 2018: Kapoor et al. 2020). Generally, yield is considered the ultimate predictor of heat tolerance of a specific cultivar (Senapati et al. 2019). Being a complex trait, yield is governed by interactions of various component traits such as biomass, number of grains and fertile tillers, etc., and the environments (Leilah and Al-Khateeb 2005; Farhad et al. 2011). Due to complex associations of heat tolerance with several quantitative traits, genetic gain has not been achieved much and breeding for heat tolerance appears to be very challenging.

Some robust, repeatable and highly reproducible markers linked to different morpho-physiological traits involved in heat tolerance are highly demanded for further improvement in wheat crops (Barik et al. 2020). QTL mapping combined with the marker-trait association can be effectively utilized for germplasm screening and identification of genetic loci, contributing to the target traits. According to Yang et al. (2002), QTL located on short arms of chromosomes 1B and 5A are linked with grain filling duration. Other studies found several QTLs for heat susceptibility indices of various morphological and yield traits on chromosomes 1A, 1B, 2A, 2B, 3B, 5A, and 6D under short-term reproductive stage heat stress (Mason et al. 2010; Mason et al. 2011). Using terminal heat stress conditions, Vijayalakshmi et al. (2010) identified QTL at loci 2A, 3A, 4A, 6A, 6B, and 7A that affect grain yield, grain weight, grain filling, stay green, and senescence-associated traits. A study was conducted on recombinant inbred lines (RILs) mapping population seeking to unravel the genetic variation for identifying potential QTLs/candidate genes for improving yield production under terminal heat stress conditions by following a three-tiered approach based on morpho-physiological phenotyping and molecular markers.

Materials and methods

The material comprised of two parents with their characteristic features and 200 recombinant inbred lines (RILs) derived from the cross between WH 711 and WH 1021 was used in the present study (Table 1). The investigation was carried out at the research farm area of Wheat & Barley Section, CCSHAU, Hisar (29°09′ N latitude; 75°43′E, longitude, 215.2 m above mean sea level and soil pH7.6) during *rabi* 2018-19 under normal (5th November) and late (10th December) sown irrigated conditions. The F_8 mapping population (RILs) were derived by following the single seed descent method from individual F_2 plants of the cross WH 711/WH 1021. Each RIL was sown in a paired row of 2.5 m length with 20 cm row-to-row distance by hand plow method with two replications in a randomized block design (RBD). Data was recorded for 31 yield attributes and morpho-physiological parameters by randomly selecting five plants of each RIL from both replications and the environments.

Meteorological parameters

Throughout the crop season, the minimum and maximum temperatures and rainfall were recorded daily (Fig. 1). The daily maximum temperature varied from 16 to 36°C and 22.9 to 38°C under timely and late sown conditions during the post-heading period. The daily minimum temperature varied
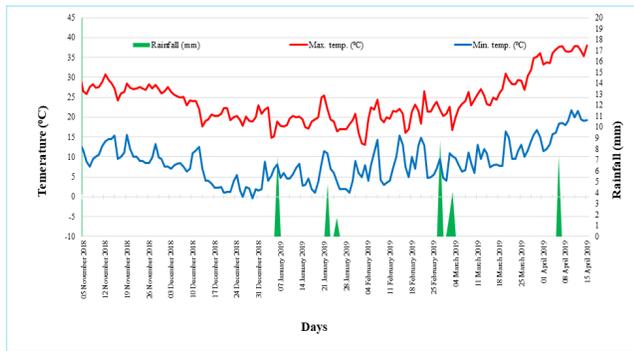
Fig. 1. Weekly maximum and minimum temperature and rainfall during *rabi* **2018-2019**

from 4 to 16.7°C and 7.4 to 21.8°C under timely and late sown conditions, respectively. The minimum average temperature (5.2°C) was recorded in January and the average maximum temperature (36.7°C) was recorded in April of 2019. During March 2019, there was only 6 mm of rainfall out of the total 44.1 mm that fell throughout the season.

Grain yield and its attributes
Yield and related traits such as, biological yield/plot (g) (BY), grain yield/plot (g) (GY/P) and harvest index (HI) were recorded. BY was recorded by weighing the complete and sun-dried harvest of the above-ground part, excluding roots, with the help of spring balance. GY/P was recorded by weighing the produce on an electronic balance after manual harvesting and threshing individual plots separately.

Morpho-physiological traits
The morphological traits included days to heading (DH), days to anthesis (DA), days to maturity (DM), grain filling duration (GFD), number of effective tillers per meter (NETM), plant height (PH), spike length (SL), number of spikelets per spike (NS/S), number of grains per spike (NG/S), 1000 grain weight (TGW), 100-grain volume (GV) and seed density (SD). The physiological parameters included normalized difference vegetation index (NDVI), canopy temperature depression (CTD), total chlorophyll content (SPAD), relative water content (RWC) (Weatherley 1950), lipid peroxidation-MDA content (Heath and Packer 1968), total antioxidant activity (TAA) (Prieto et al. 1999), gas exchange parameters viz., photosynthetic rate (A), transpiration rate (E), intercellular CO_2 concentration (Ci) and stomatal conductance (gs), carboxylation capacity (Cc), instantaneous water-use efficiency (WUE), intrinsic water-use efficiency (WUEi). Normalized difference vegetative index (NDVI 1), canopy temperature depression (CTD 1) and total chlorophyll content (SPAD 1) (°C) were recorded at the anthesis stage. In contrast, normalized difference vegetative index (NDVI 2), canopy temperature depression (CTD 2) and total chlorophyll content (SPAD 2) (°C) were measured at 15 DAA stage. NDVI, CTD, SPAD readings were measured

at anthesis and 15 days after the anthesis stage by using Green Seeker, infrared thermometer and SPAD chlorophyll meter, respectively. Gaseous parameters were measured at anthesis with an infrared gas analyzer (IRGA, LCi-SD, ADC biosciences). The average of all these parameters was recorded and subjected to analysis of variance (ANOVA) (Panse and Sukhatme 1954). Relative water content (RWC) in percent, malondialdehyde content (MDA) (μmole/g fresh wt.) in percent, total antioxidant activity (TAA) (μmole/g fresh wt.), internal CO_2 concentration (Ci) (μmol/mole of air), transpiration rate (E) (mmol m^{-2}s^{-1}), stomatal conductance (gs) (mol m^{-2} s^{-1}) and photosynthetic rate (A) (μmol m^{-2} s^{-1}) were recorded at anthesis stage. In addition, carboxylation capacity (Cc), instantaneous water use efficiency (WUE) and Intrinsic water use efficiency (WUEi) were also recorded.

Genetic diversity and QTLs mapping
Total genomic DNA of 200 RILs along with parents, was extracted using CTAB (Cetyl Trimethyl Ammonium Bromide) extraction method from 35-day-old seedlings (Murray and Thompson, 1980). A total of 185 simple sequence repeats (SSRs) markers were utilized to investigate molecular polymorphism among parents. Out of 185, 24 polymorphic SSRs (Supplementary Table S1) were found and further used for amplification of genomic DNA of RILs. Distinct bands for SSR were visually scored and recorded as binary data matrices with the presence or absence (read as one and zero, respectively) of the band. A sub-program of Numerical Taxonomy System for Personal Computer (NTSYS-pc) v2.02 'simqual' was employed to generate a dendrogram using the un-weighted pair-group method with arithmetic average (UPGMA) (Rohlf and Slice 1993). Furthermore, the reliability of the dendrogram was ensured by 2-D and 3-D scatter plots. The Windows QTL cartographer v. 2.5 (Wang, 2007) was used for QTL analysis and to determine their respective locations and effects using the composite interval mapping (CIM) method. A threshold of P ≤ 0.005 and LOD ≥ 2.5 was used to identify significant QTLs and to create the linkage maps. The QTLs were named by combining the letter *Q*, *trait abbreviation*, *research department* and *chromosome* (Zhou et al. 2005). The graphical genotyping (Young and Tanksley 1989) of RILs was performed using GGT v. 2.0 for selection, evaluation and depiction of the parent's genome recovery among RILs.

Results

Variability analysis of grain yield, its attributes and morpho-physiological traits
Under timely sown and late sown environments, ANOVA revealed highly significant variances due to genotypes (*p* < 0.01) and non-significant variances due to replications for 31 traits. The mean sum of squares (Table 2) indicated a high magnitude of genotypic variability among RILs for all
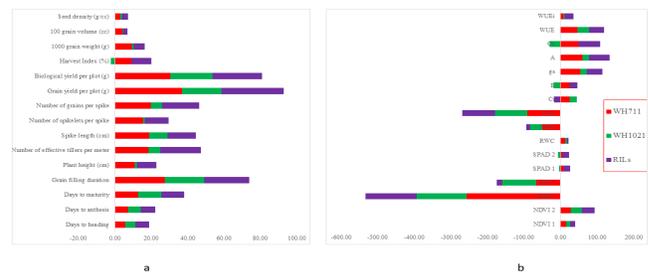
a

d
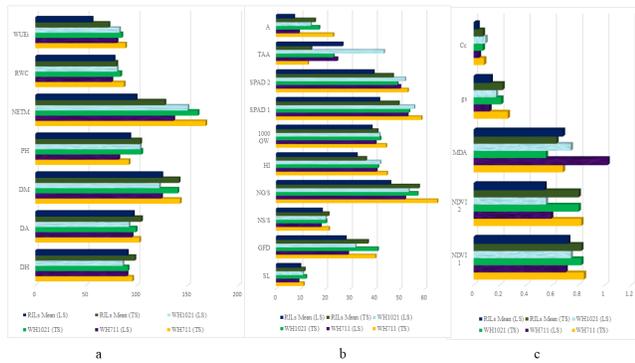
Fig. 2. Mean performance of parents and RILs for various yield attributes and morpho-physiological traits under timely and late sown conditions (A to F)

a

b

Fig. 3. Per cent reduction in morphological (a) and physiological (b) traits in parents and RILs

31 traits. The coefficient of variation (CV) ranged from 1.04% (GFD) to 10.88% (CTD 1) and 1.70% (NDVI 2) to 10.89 (CTD 1) under timely and late-sown conditions, respectively.

Analysis of phenotypic performances

A decline was observed for all the studied traits under heat stress conditions while compared to those under normal conditions (Supplementary Table S2 and Fig. 2). Lower percent reduction was observed in GY/P and its component traits of WH1021 as compared to WH711 under heat stress conditions (Fig. 3). For WH1021, GY/P was recorded 905g and 710gunder timely and late sown conditions respectively, a 21.54% drop (Figs. 2 and 3). In contrast, GY/P for WH711 was 1050g and 660g under timely and late sown conditions, respectively and a 37.14% drop. Likewise, a similar scenario was observed in GY/P component traits like NETM, SL, PH, NS/S, NG/S, BY, TGW, SD, NDVI 1, RWC, Ci, A, gs, WUEi and WUE (Figs. 2 and 3). The traits, HI, SPAD 1, SPAD 2, E and Cc for WH1021 and CTD 1, CTD 2, MDA and TAA for parents as well as RILs, were positively scored under terminal heat stress conditions. GFD was observed 24 to 43 and 18 to 35 days, while PH was 86 to 117 and 71 to 107 cm under timely and late sown conditions, respectively. The heat stress causes a sharp decline in yield attributes, i.e., GYP (from 540–1220 g to 217–900 g) and BY (from 1450–3500 g to 900–2450 g).

The gaseous parameters measured on the flag leaf at the time of anthesis ranged from 9.57 to 25.08 µmole m^{-2}s^{-1}, 3.0 to 7.9 mmol m^{-2}s^{-1} and 0.11 to 0.37 mol m^{-2}s^{-1} under timely sown conditions, whereas 3.21 to 21.69 µmolem^{-2}s^{-1}, 0.23 to 6.9 mmol m^{-2}s^{-1} and 0.07 to 0.25 mmol m^{-2}s^{-1}under late sown conditions, respectively. A negative and lowest reduction was observed in CTD 1, TAA, CTD 2 and MDA, whereas the maximum reduction was observed in A, WUE, gs, GY/P and BY.

Genetic relationship by dendrogram and PCA analysis

The amplified profile of two SSR markers (Xgwm448 and Xcfd 2121) was shown in Supplementary Figure S1. The dendrogram utilized allelic diversity from SSR markers to signify genetic linkage among parents and their RILs. The dendrogram classified the whole population into two major groups A (cluster I) and B (cluster II) at the similarity coefficient of 0.22 (SupplemetaryFigure S2). Cluster I consisted of only donor parent WH1021, while Cluster II consisted of recipient parent WH711, along with 200 RILs. Cluster II was further categorized into sub-cluster II-A and sub-cluster II-B, with 2 RILs at a similarity coefficient of 0.54. The sub-cluster II-B was further sub-categorized into two groups *viz.,* the first group with only 1 RIL (102).On the other hand, sub-cluster II-A was composed of the rest of the RILs along with recipient parent WH711.

Additionally, NTSYS-PCA 2-D and 3-D scaling were employed(a) to validate the dendrogram results and (b) to assess the genetic relationships of RILs with parents (Figure 4[a and b]). Similar to the dendrogram outcome, both 2-D and 3-D PCA scaling also exhibited dissimilarity between two parents and RILs were distributed between two parental lines. The progenies (RILs 102, 81, 37, 36, 101, 89, 117, 90, 35, 79, 70, 58, 55, 169, 182, 162, and 185) were found closer to WH1021, indicated their predominant heat tolerance. On the other hand, the RILscloser to WH711 indicated their predominant heat susceptibility. The results were significantly consistent with the dendrogram.

Table 2. ANOVA for different morpho-physiological parameters in RILs of the cross WH711/WH1021 under timely sown conditions

Traits	Timely sown				Late sown			
	Replication	Genotype	Error	CV (%)	Replication	Genotype	Error	CV (%)
DH	6.76	26.64**	7.46	2.05	9.61	67.41**	17.2	3.019
DA	6.25	23.15**	7.64	1.87	17.22	64.82**	19.22	3.019
DM	0.56	9.37**	3.37	1.93	3.422	11.13**	4.34	3.019
GFD	3.06	40.34**	15.73	7.52	5.29	58.16**	20.94	1.049
PH	35.192	93.77**	36.57	5.24	0.47	95.89**	28.77	8.779
NETM	13.69	744.31**	230.74	9.67	29.16	947.57**	198.99	9.654
SPL	0.087	2.72**	1.17	6.74	0.93	2.59**	0.88	7.968
NS/S	3.422	12.37**	4.08	6.01	2.89	10.13**	4.15	5.789
NG/S	6.76	116.76**	43.200	8.26	10.24	121.18**	42.41	6.857
GY/P	1176.49	60715.13**	20035.99	9.83	976.56	35859.07**	10757.72	9.953
BY	3025.00	272961.3**	98806.86	9.52	62001	269835.1**	86347.23	9.312
HI	1.193	30.72**	12.6	7.67	5.664	31.92**	10.85	7.542
TGW	4.823	22.80**	5.93	7.22	6.708	25.23**	5.8	6.538
GV	0.003	0.29**	0.073	6.06	0.028	0.275**	0.065	4.168
SD	0.003	0.042**	0.013	6.12	0.001	0.034**	0.009	5.722
NDVI1	0.001	0.005**	0.0015	6.00	0.0001	0.002**	0.0004	1.84
NDVI2	0.001	0.0124**	0.0014	1.70	0.0001	0.002**	0.0004	1.83
CTD1	3.152	8.95**	2.94	10.89	0.598	1.996**	0.252	10.88
CTD2	0.281	5.79**	0.567	10.14	0.325	3.16**	0.39	10.59
SPAD1	10.498	64.81**	24.07	10.11	0.0001	38.86**	4.02	2.25
SPAD2	0.226	119.05**	18.24	4.33	2.102	35.41**	4.19	2.51
RWC	2.324	50.55**	7.50	2.51	4.744	42.28**	6.47	2.25
MDA	0.013	0.28**	0.077	10.08	0.002	0.046**	0.015	10.13
TAA	4.757	189.74**	25.97	10.45	0.765	79.12**	10.47	7.96
Ci	164.02	10137.9**	2249.19	1.71	80.103	1654.84**	741.73	9.82
E	0.022	3.021**	0.622	2.27	0.0001	3.39**	0.54	10.08
gs	0.001	0.0028**	0.0004	5.83	0.001	0.004**	0.00124	10.31
A	0.044	25.33**	2.67	3.55	0.616	25.38**	4.70	9.77
Cc	0.0001	0.0005**	0.00011	9.22	0.0004	2.65**	0.0002	10.35
WUE	0.0001	2.46**	0.768	6.51	0.24	2.66**	0.043	10.43
WUEi	9.063	729.26**	95.62	6.25	138.33	223.33**	89.90	10.375

**Significant at ($p < 0.01$) per cent LSD

Graphic genotyping using SSR markers

The graphical genotyping revealed that the genome recovery of WH711 ranged from 29% (RIL 102) to 100% (RILs 110 and 113), while for WH1021, genome recovery was observed from 0 (RILs 14, 29, 63, 64, 67, 85, 91, 110, 113, 123 and 140) to 71% (RIL 102) (data not shown). The average recovery of parent WH711, WH1021 and recombinants were 76.5 and 16.4 and 3.3%, respectively. The RILs, *i.e.,* 44, 59, 102 and 193, recovered more than 65% genome of heat-tolerant parent WH1021.
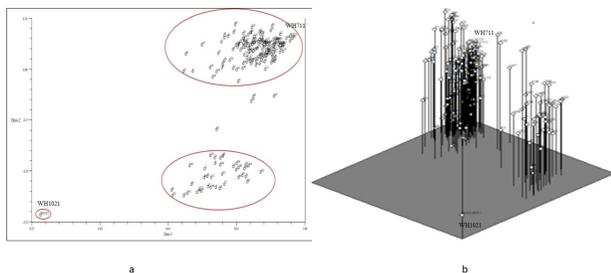
Fig. 4. 2-D (a) and 3-D (b) PCA scatter plots showing genetic relationships among parents and their RILs

QTL analysis using CIM

CIM detected a total of seven putative QTLs for various yield attributes and morpho-physiological traits under both conditions (Table 3 and Figure 5). The LOD scores of respective QTLs varied from 2.92 (*Qnsps.ccshau-3B*) to 7.08 (*Qgypp.ccshau-1B*) and explained 0.35 to 12.92% of the phenotypic variability.

QTLs associated with yield and its contributing attributes

Two QTLs, namely *Qgypp.ccshau-1B* and *Qgypp.ccshau-7A* flanked by the markers Xgwm364 (18.0 cM) and Xwmc596 (72.2 cM) were mapped on chromosome 1B and 7A at a mapping distance of 11.01cM and 2.71cM with a LOD value of 2.92 and 5.48 under timely and late sown experiments, respectively. Both QTLs showed significantly higher additive effects (22.33 and 54.88) and phenotypic variation (R^2) 0.35% and 12.92% under timely and late-sown conditions, respectively. The QTL *Qbypp.ccshau-7A* flanked by the markers Xwmc596 (72.2 cM), was mapped for BY under late sown conditions. This showed a maximum positive additive value (90.34) with a 3.35 value of LOD score and significant phenotypic variation (R^2) of 7.37%.
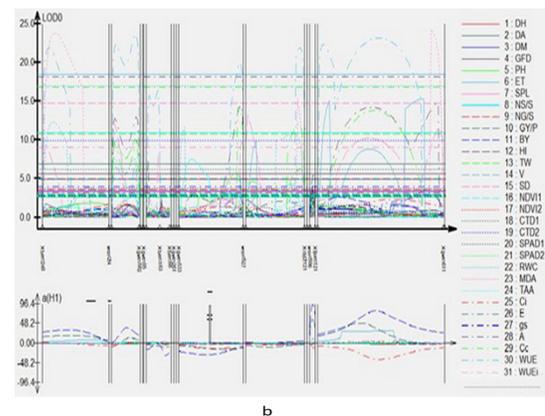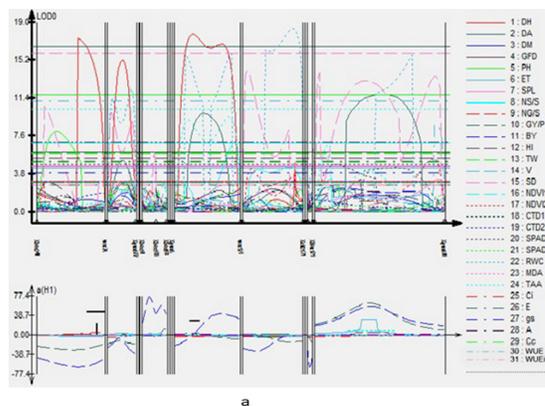
Fig. 5. Likelihood curve of LOD scores of QTLs mapped for yield attributes and morpho-physiological trait under timely (a) and late (b) sown conditions

QTLs associated with morphological traits

The QTL (*Qnsps.ccshau-3B*) in the interval of markers Xgwm533-Xwmc527, was mapped on chromosome 3B for NS/S at a mapping distance of 41.01 cM under

Table 3. Identification of QTLs for yield attributes and morpho-physiological traits in RILs of the cross WH711/WH1021 based on CIM using QTL Cartographer

Trait	QTL name	Chromosome	Position (cM)	Flanking marker	Flanking marker position	LOD	Additive	R^2	Trial
Yield attributes									
GY/P (g)	*Qgypp.ccshau-1B*	1B	11.01	Xgwm364	18	2.92	22.328	0.35	TS
GY/P (g)	*Qgypp.ccshau-7A*	7A	2.71	Xwmc596	72.2	5.48	54.876	12.92	LS
BY (g)	*Qbypp.ccshau-7A*	7A	2.71	Xwmc596	72.2	3.35	90.333	7.37	LS
Morphological traits									
NS/S	*Qnsps.ccshau-3B*	3B	41.01	Xgwm533-Xwmc527	26.0-71.2	7.08	0.112	1.88	TS
GV (cc)	*Qgv.ccshau-1B* 0.1	1B	3.01	Xgwm364	18	6.53	0.0133	1.79	LS
GV (cc)	*Qgv.ccshau-1B* 0.2	1B	17.01	Xgwm364	18	6.4	-0.0245	4.81	LS
Physiological traits									
NDVI 1	*Qndvi-ccshau-7A*	7A	2.71	Xwmc596	72.2	3.13	0.012	5.07	LS

TS= Timely sown, LS= Late sown, LOD= Likelihood of odd ratio, NS/S= Number of spikelets/spike, GY/P= Grain yield/plot (g), BY= Biological yield/plot (g), GV= 100 grain volume, NDVI 1= Normalized difference vegetative index at anthesis stage, R^2= Percentage of phenotypic variance showed by QTL

timely sown conditions. The QTL explained 1.88% of the phenotypic variance with 7.08 LOD value and a smaller positive (0.112) additive effect. Two QTLs (*Qgv.ccshau-1B* 0.1 and *Qgv.ccshau-1B* 0.2) associated with GV were present on chromosome 1B at a map distance of 3.01 and 17.01 cM and LOD value of 6.53 and 6.4, explained 1.79 % and 4.81% of phenotypic variance under late sown conditions, respectively.

QTLs associated with physiological traits

One QTL, *Qndvi-ccshau-7A* flanked by marker Xwmc596 was identified. The QTL was associated with NDVI 1 and was mapped on chromosome 7A at a mapping distance of 2.71 cM with a LOD value of 3.35. It showed the lowest positive additive effect (0.01) and reflected significant phenotypic variation (R^2) of 5.07%.

Discussion

Screening of different morpho-physiological traits under late-sown conditions is an effective approach for selecting genotypes for heat tolerance (Agarwal et al. 2021). The ANOVA results showed that the mean sum of the square was highly significant for genotypes (Table 2), indicating the presence of an ample amount of genetic variation among RILs, which can be utilized for a selection of superior and desired genotypes for further improvement. The lowest coefficient of variations for GFD indicated shrinkage of RILs dispersion level in terms of grain filling period, whereas the maximum level of dispersion was recorded for CTD 1indicating wide response of RILs in terms of cooling efficiency under heat stress conditions. Previous studies reported significant wide variability for wheat grain yield and its component traits (Bhutto et al. 2016; Farooq et al. 2011; Mohammadi et al. 2012). A significant mean sum of squares was reported in various studies for different morpho-physiological traits due to genotypes (Arya et al. 2017; Bhusal et al. 2017; Pooja 2018).

During the pre-heading phase, wheat crops experienced favorable environmental conditions in terms of proper moisture and continuous declining temperature, which played a crucial role in vernalization phenomena. However, the reproductive phase faced continuous high-temperature stress (Fig. 1). A positively accelerated linear trend was observed in temperature in mid-February, which remained continuous and bypassed 30°C at the end of March. Most of the morpho-physiological parameters showed reduction under heat stress conditions except some physiological parameters *viz*. CTD 1, CTD 2, MDA, and TAA showed higher values under stress conditions. The recorded higher reduction percent for gaseous parameters, GY/P, BY and GFD, signified their high proneness towards heat stress. Among grain yield components, the highest range was observed for BY, followed by the NETM, Ci and WUEi under timely and late sown conditions, indicating differential response of RILs under heat stress conditions.

The similarity matrix recorded the wide range of similarity coefficients for RILs and parents, indicating a wide spectrum of genetic divergence at the genotypic level among parents and the RILs population. The genetically diverse nature of parents can also be confirmed by their respective pedigrees, which are completely different from each other. The UPGMA tree analysis grouped the whole RILs progenies in two separate groups, indicating the higher inclination of the genetic background of the RILs population towards agronomic superior parent WH711. No RIL was found with similar heat tolerance as the heat tolerant parent had. The smaller value of the similarity coefficient also validates our selection practices as we promoted only heat tolerant and high-yielding RILs progenies under heat stress environments every year. Saha et al. (2020) categorized progeny into different groups at a similarity coefficient of 0.33. Similar results are also reported by Al-Tamimi and Al-Janabi (2019), who stated that all the wheat genotypes were clustered into one large cluster except a single cultivar, *i.e.*, Tamuz-2. Sareen et al. (2020) broadly clustered four hundred indigenous and ninety-six exotic wheat accessions into two major groups: heat tolerant and heat susceptible.

According to 2-D and 3-D PCA scaling results, both parents were found located on the very far end of plots, depicting their diverse nature, whereas RILs were found closely located to each other, showing close ties of their consanguinity. Broadly, they were categorized into two groups *i.e.*, (a) towards WH711 and (b) towards WH1021, which can also be termed as heat susceptible and heat tolerant groups, respectively. Sharma et al. (2017) also divided wheat genotypes into heat-tolerant and susceptible groups. Visualization of marker data can play an important role in selection for individuals containing positive genome regions or, reversely, against individuals that are carrying negative genome regions. The results of graphical genotyping revealed higher genome recovery of WH711 as compared to WH1021 in the RILspopulation, denoting the crucial role of the mother parent in a breeding population due to cytoplasmic inheritance. These results validated the results of the UPGMA cluster dendrogram, which clearly indicated a high proportion, including phenotypic traits of agronomic superior parent WH711 and low proportion (especially heat tolerance) of heat tolerant parent WH1021 in the genetic constitution of RILs.Better performance was recorded for RILs 44, 59 and 102 under heat stress, with more than 65% recovery of heat-tolerant parent WH1021. Therefore, these RILs may be utilized in future heat-tolerance breeding programs.

This study identified a total of seven QTLs associated with mean phenotypic data of NS/S, GV, GYPP, BYPP and NDVI 1 over both conditions. Many researchers reported a well-known relationship between grain yield and its component traits with QTLs, which are highly affected by environmental factors (Babita et al. 2018). The first QTL (*Qnsps.ccshau-3B*)

was for the NS/Son chromosome 3B, explained 1.88% of phenotypic variance and showed a high LOD score, which made it a major QTL. Generally, the higher number of spikelets leads to the production of a higher number of grains and, thus, increases grain yield. In addition to it, two novel QTLs were identified for GV (*Qgv.ccshau-1B* 0.1 and *Qgv. ccshau-1B* 0.2) on chromosome 1B, were found associated with marker Xgwm364 under late sown conditions. Pinto et al. (2010) reported 3B region as one of the most important chromosome regions in the context of heat tolerance, which was found to be associated with several heat stress traits like yield, canopy temperature, height, grain number and thousand-grain weight. In the context of heat tolerance, the GV becomes even more crucial as it is closely linked to the grain-filling process, which is highly sensitive to elevated temperatures. Identifying a novel QTL associated with GV under heat stress conditions is of great scientific importance as it provides a specific genetic target for improving grain size and weight in heat-tolerant wheat cultivars. There are very limited QTLs studies associated with grain volume, therefore, this could be a good guide for future studies.

Several yield QTLs were reported on chromosome 1B in previous studies; therefore, it should be considered an important site of yield QTLs(Rehman Arif et al. 2020). To add to it, two QTLs were discovered in our study for GY/P, one under timely sown condition (*Qgypp.ccshau-1B*) and another under late sown conditions (Qgpp.ccshau-7A). Along with this, one QTL was also identified for BY (Qbypp.ccshau-7A) located on chromosome 7A.*Qgpp.ccshau-7A* and *Qbypp. ccshau-7A* were associated with GY/P and BY, one of the most studied heat tolerance traits in conventional breeding. Thus, these QTLs can be of utmost importance. The positive additive effect of QTLs revealed that the allele for the WH1021 should be considered for heat tolerance and *vice-versa*. The results are similar to the findings of Vijayalakshmi et al. (2010); Paliwal et al. (2012); Mason et al. (2013) and Rehman Arif et al. (2020), who reported QTLs associated with grain yield component traits on chromosome 2D, 3B, 5D, 7B and 7D under heat stress. However, one major QTL(Qndvi-ccshau-7A), located on chromosome 7Awas found to be associated with NDVI 1and thus, highlights the crucial role of NDVI1 in heat tolerance. Shi et al. (2017)and Sangwan et al. (2019) identified QTL associated with stay green (NDVI) and agronomic traits 1B and 7A chromosomes. Many previous studies reported association of chromosome 7A with heat tolerance (Mason et al. 2013; Talukder et al. 2014)

Overall, it can be stated that three genomic regions (chromosome 1B, 3B and 7A) were found to be significantly associated with seven QTLs linked with grain yield and its attributes, which ultimately turn into higher grain yield collectively. Guan et al.(2018) detected QTL on chromosomes 1B, 2A, 2B, 3B, 5A and 7A in a double haploid population for heat tolerance under hot conditions. Previous research has

identified QTLs linked to heat tolerance traits, such as grain yield, biomass, and canopy temperature, in the vicinity of the marker Xgwm364 (18.0 cM) flanking the QTL Qgypp. ccshau-1B. The QTL Qnsps.ccshau-3B associated with the NS/S is located on chromosome 3B. While this specific QTL might be less frequently reported in other heat tolerance studies, chromosome 3B as a whole has been found to harbor several QTLs related to heat tolerance traits. Previous studies have identified QTLs on this chromosome for traits like thousand-grain weight, grain yield, and canopy temperature under heat stress conditions. The QTL Qgypp. ccshau-7A associated with grain yield and the QTL Qndvi-ccshau-7A associated with NDVI are located on chromosome 7A. Chromosome 7A has been widely studied for heat tolerance traits in wheat, and several studies have reported QTLs associated with grain yield, canopy temperature, and other agronomic traits on this chromosome. The presence of QTLs on chromosome 7A in this study confirms its relevance in imparting heat tolerance to bread wheat. Overall, the identification of QTLs in the genomic regions 1B, 3B, and 7A in this study, in agreement with previous findings from various heat tolerance studies, strengthens the reliability and significance of the results.

Supplementary material
Supplementary Table S1 to S2 and Supplementary Figs. 1 and 2 are provided, which can be accessed at www.isgpb.org

Authors' contribution
Conceptualization of research (VS, SY); Designing of the experiments (VS, SY, MR); Contribution of experimental materials (VS); Execution of field/lab experiments and data collection (MR, KS); Analysis of data and interpretation (MR, KS, SK); Preparation of manuscript (MR, VS, SY, KS).

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Supplementary Table S1. Microsatellite markers used for molecular screening

| S. No.	SSR Primers	Forward Sequence (5'-3')	Reverse Sequence (5'-3')	Annealing temperature (°C)	Chromosome location
1	Xcfd2121	TAAATGGCCATCAAGCAATG	GCTTGTGAACTAATGCCTCCC	58	4A
2	Xwmc24	GTGAGCAATTTTGATTATACTG	TACCCTGATGCTGTAATATGTG	51	1A
3	Xwmc527	ACCCAAGATTGGTTGCAGAA	GCTACAGAAAACCGGAGCCTAT	55.8	3B
4	Xwmc596	TCAGCAACAAACATGCTCGG	CCCGTGTAGGCGGTAGCTCTT	58.7	7A
5	Xwmc603	ACAAACGGTGACAATGCAAGGA	CGCCTCTCTCGTAAGCCTCAAC	58.6	7A
6	Xbarc10	GCGTGCCACTGTAACCTTTAGAAGA	GCGAGTTGGAATTATTTGAATTAAACAAG	52	2B
7	Xbarc98	CCGTCCTATTCGCAAACCAGATT	GCGGATATGTTCTCTAACTCAAGCAATG	55	2B
8	Xbarc121	ACTGATCAGCAATGTCAACTGAA	CCGGTGTCTTTCCTAACGCTATG	56.1	7A
9	Xbarc181	CGCTGGAGGGGGTAAGTCATCAC	CGCAAATCAAGAACACGGGAGAAAGAA	60	1B
10	Xbarc183	CCCGGGACCACCAGTAAGT	CGGGGAATTGGAGATACAGAG	58	2B
11	Xbarc240	AGAGGACGCTGAGAACTTTAGAGAA	GCGATCTTTGTAATGCATGGTGAAC	52	1A
12	Xgwm5	GCCAGCTACCTCGATACAACTC	AGAAAGGGCCAGGCTAGTAGT	57.5	3A
13	Xgwm95	GATCAAACACACACCCCTCC	AATGCAAAGTGAAAAACCCG	60	2A
14	Xgwm261	CTCCCTGTACGCCTAAGGC	CTCGCGCTACTAGCCATTG	56	2D
15	Xgwm292	TCACCGTGGTCACCGAC	CCACCGAGCCGATAATGTAC	56.4	5D
16	Xgwm364	ATCACAATGCTGGCCCTAAAAC	CAGTGCCAAAATGTCGAAAGTC	56	1B
17	Xgwm429	TTGTACATTAAGTTCCCATTA	TTTAAGGACCTACATGACAC	46.6	2B
18	Xgwm448	AAACCATATTGGGAGGAAAGG	CACATGGCATCACATTTGTG	60	2A
19	Xgwm533	AAGGCGAATCAAACGGAATA	GTTGCTTTAGGGGAAAAGCC	53.3	3B
20	Xgwm540	TCTCGCTGTGAAATCCTATTTC	AGGCATGGATAGAGGGGC	55	7B
21	Xgwm577	ATGGCATAATTTGGTGAAATTG	TGTTTCAAGCCCAACTTCTATT	48.7	7B
22	Xgwm582	AAGCACTACGAAAATATGAC	TCTTAAGGGGTGTTATCATA	52	1B
23	Xgwm601	ATCGAGGACGACATGAAGGT	TTAAGTTGCTGCCAATGTTCC	60	4A
24	Xgwm611	CATGGAAACACCTACCGAAA	CGTGCAAATCATGTGGTAGG	55	7B
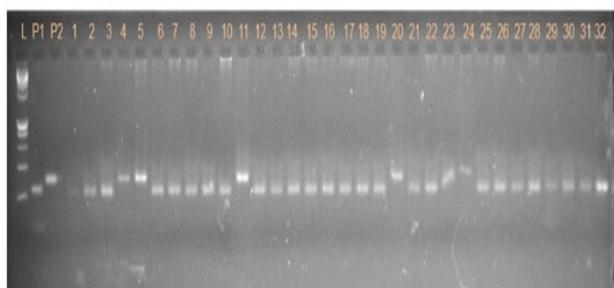
(a) Xgwm 448

(b) Xcfd 2121

Supplementary Fig. 1. SSR markers profiles of parents and RILs population using (a)Xgwm 448 (b)Xcfd 2121 SSR primers

Supplementary Table S2. Mean of parents and recombinant inbred lines (RILs) and range of RILs for different morpho-physiological traits under timely and late sown conditions

S. No.	Traits	WH711		WH1021		RILs Mean ± SE(m)		RILs Range	
		Timely sown	Late sown	Timely sown	Late sown	Timely Sown	Late Sown	Timely Sown	Late Sown
1.	Days to heading	95.50	90.00	91.00	86.00	97.74 ± 0.55	90.29 ± 0.28	86-110	81-99
2.	Days to anthesis	102.50	95.00	99.00	92.00	104.27 ± 0.52	96.24 ± 0.29	91-116	85-105
3.	Days to maturity	142.50	124.00	140.00	122.00	141.28 ± 0.18	124.27 ± 0.14	136-147	120-130
4.	Grain filling duration	40.00	29.00	41.00	32.00	37.01 ± 0.81	28.03 ± 0.56	24-43	18-35
5.	Plant height (cm)	92.00	81.93	104.50	103.07	103.49 ± 1.82	92.77 ± 2.32	86-117	71.67-107.67
6.	Number of effective tillers per meter	167.50	136.00	160.00	150.00	127.28 ± 1.82	99.00 ± 2.32	76-204	62-160
7.	Spike length (cm)	11.11	9.00	12.11	10.87	11.49 ± 0.31	9.75 ± 0.29	8.4-15	7.0-13.0
8.	Number of spikelets per spike	21.33	18.00	20.18	20.00	21.13 ± 0.31	18.42 ± 0.36	15-23	13-21
9.	Number of grains per spike	65.00	52.00	57.00	53.50	57.57 ± 0.93	46.02 ± 0.83	40-83	27-69
10.	Grain yield per plot (g)	1050.00	660.00	905.00	710.00	855.05 ± 4.43	563.99 ± 4.50	540-1220	217-900
11.	Biological yield per plot (g)	2350.00	1630.00	2200.00	1690.00	2369.75 ± 7.02	1731.00 ± 5.59	1450-3500	900-2450
12.	Harvest Index (%)	44.68	40.49	41.14	42.01	36.25 ± 0.57	32.47 ± 0.64	30.27-46.00	22.15-42.38
13.	1000 grain weight (g)	44.32	40.10	42.01	41.67	40.87 ± 0.53	38.48 ± 0.49	27.52-46.00	25.2-42.0
14.	100 grain volume (cc)	3.40	3.25	3.36	3.35	3.19 ± 0.20	3.12 ± 0.20	2.2-4.3	1.6-4.2
15.	Seed density (g/cc)	1.27	1.23	1.25	1.24	1.29 ± 0.11	1.25 ± 0.11	1.00-1.93	0.92-1.84
16.	NDVI 1	0.84	0.70	0.82	0.74	0.82 ± 0.03	0.72 ± 0.04	0.71-0.89	0.53-0.84
17.	NDVI 2	0.82	0.59	0.80	0.55	0.80 ± 0.03	0.54 ± 0.10	0.70-0.86	0.30-0.72
18.	CTD 1	1.26	4.50	2.30	5.47	2.56 ± 0.60	6.08 ± 0.78	0.00-4.75	0.00-10.00
19.	CTD 2	1.50	2.50	3.50	6.75	4.19 ± 0.60	4.78 ± 0.76	0.10-6.50	0.00-7.00
20.	SPAD 1	58.60	53.00	53.66	55.68	49.41 ± 0.62	41.55 ± 0.67	40.00-60.90	21.2-61.5
21.	SPAD 2	53.10	50.00	48.95	52.00	47.09 ± 0.60	39.32 ± 1.22	34.50-55.80	21.1-57.5
22.	RWC	87.24	75.10	83.87	80.43	79.92 ± 0.50	77.26 ± 0.45	59.87-85.18	50.82-82.96
23.	MDA	0.68	1.02	0.55	0.74	0.63 ± 0.18	0.68 ± 0.45	0.15-1.13	0.15-2.38
24.	TAA	12.86	24.66	23.18	43.48	14.21 ± 1.66	26.79 ± 1.81	5.11-30.75	10.10-45.20
25.	Ci	288.00	215.00	247.50	200.00	243.07 ± 1.49	285.62 ± 2.51	178.00-336.00	153-393
26.	E	5.20	3.89	4.90	5.84	5.20 ± 0.55	4.15 ± 0.61	3.00-7.99	0.23-6.99
27.	gs	0.26	0.12	0.21	0.17	0.22 ± 0.09	0.13 ± 0.10	0.11-0.37	0.07-0.25
28.	A	23.04	9.20	17.39	14.05	15.70 ± 0.86	7.26 ± 1.32	9.57-25.08	3.21-21.69
29.	Cc	0.08	0.04	0.07	0.09	0.07 ± 0.06	0.03 ± 0.10	0.03-0.13	0.01-0.14
30.	WUE	4.43	2.37	3.55	2.41	3.22 ± 0.62	1.94 ± 1.06	1.44-7.89	0.65-6.49
31.	WUEi	88.62	80.15	84.88	82.65	72.21 ± 1.08	55.61 ± 2.54	33.34-91.71	20.11-93.80

NDVI 1: Normalized difference vegetative index at anthesis stage, NDVI 2: Normalized difference vegetative index at 15 DAA stage, CTD 1: Canopy temperature depression at anthesis stage ($^{\circ}$C), CTD 2: Canopy temperature depression at 15 DAA stage ($^{\circ}$C), SPAD 1: Total chlorophyll content at anthesis stage,SPAD 2: Total chlorophyll content at 15DAA stage, RWC : Relative water content at anthesis stage (%), MDA: Malondialdehyde content at anthesis stage (μmole/g fresh wt.), TAA : Total antioxidant activity at anthesis stage (μmole/g fresh wt.),Ci : Internal CO_2 conc. at anthesis stage (μmole/mole of air),E: Transpiration rate (mmol m^{-2}s^{-1}) at anthesis stage, gs : Stomatal conductance (mol m^{-2} s^{-1})at anthesis stage,A :Photosynthetic rate (μmole m^{-2} s^{-1}) at anthesis stage, Cc : Carboxylation capacity,WUE : Water use efficiency, WUEi : Intrinsic water use efficiency
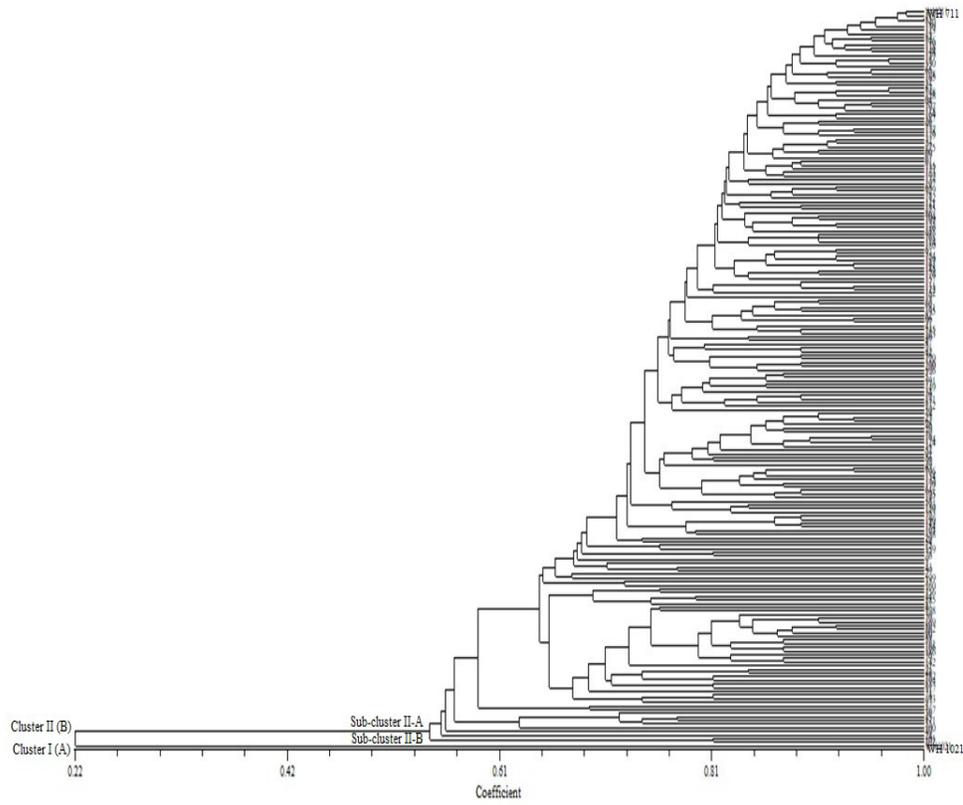
Supplementary Fig. 2. Cluster dendrogram showing genetic linkage among parents and their RILs based on SSR allele data