Mapping QTLs for physiological traits associated with grain yield under timely and delayed sown conditions in wheat (*Triticum aestivum* L.)

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Abstract

Twenty-one quantitative trait loci were discovered across six environments, including three (NS), seven (LS), and eleven (VLS) conditions. The linkage groups 1A, 1B, 2A, 2B, 2D, 3B, 4A, 6A, 6B, and 7D spanned QTLs. For CT, a stable QTL (*Qct_rpcau_4A*) in the environment E6 has shown a PVE up to 17.85%. The QTL, *Qspad_rpcau_2D* was found to be stable for SPAD value, explaining the PVE of 15.05%. Whereas, three stable QTLs viz. *Qgy_rpcau_3B*, *Qgy_rpcau_4A* and *Qgy_rpcau_6A* were associated with the chromosomes 3B, 4A and 6B explaining a PVE of 20.85%, 17.43% and 17.37% respectively for the trait GY. The interaction of genotype and environment has been shown to be useful in determining the best lines for heat stressed environments. In addition, G X E analysis was performed on the population. In the ranking of genotypes for both mean yield and mean Vs. stability, GGE biplot across the six environments, DH 146, 195, 60, 202, 65, 155, 124, 5, 201, 169, 15, and 170 were ranked closest to ideal and winning genotype; these were highly adapted and most stable lines. The goal of the current investigation was to find the best suitable double haploid lines which can withstand heat-stress and to find those QTLs which are influential to heat stress for the studied traits.

Introduction

Cereals serve a critical role in satisfying global food demand and ensuring food security. Wheat is produced on 217 million hectares with a global output of 731 million tonnes (<http://www.fas.usda.gov>) and occupies a vast agricultural area of 30.54 million hectares with a national production of 94.57 million tonnes¹ (Ramadas et al., 2019). It provides edible grain which forms staple food for billions of people world over² (Verma et al. 2020). The latest United Nations projections indicate that world population will reach 10 billion in the year 2056³ (Prakash et al. 2019). Climate change-related extinction is a serious restriction in wheat-growing regions. It is prone to heat stress which affects its production on a large Scale⁴ (Pankaj et al. 2021). Over a decade, global wheat output has decreased by up to 5% while temperatures have risen by 0.13 ° C⁵. (Hatfield et al., 2017). According to Asseng et al. 2015, each 1°C increase in temperature to 23°C would cut world wheat yield by 6%⁶. During the rabi season in 2020, south asian regions will have maximum and lowest temperatures of 1.54°C and 1.08°C, respectively⁷ (Bhusal et al., 2017). Wheat output in India will be reduced by 4 million tonnes for every 1°C increase in temperature. High temperatures have an impact on more than 40% of the world's wheat-growing region. Wheat yield is affected by high temperatures, either as a consequence of chronic stress at 32°C or by rapid heat shock, although only for a short period of time up to 33°C and higher. Wheat yields are being impacted by global warming. Heat waves in the United States and Canada, as recorded lately, may have an influence on crops. Heat waves have become increasingly powerful, frequent, and long-lasting, and as global warming continues these trends are expected to worsen. At the morphological, physiological, biochemical, and molecular levels, high temperatures have a profound impact. High temperatures around critical plant growth stages, such as flowering and grain filling, can significantly reduce grain yields⁸ (Ali et al., 2021), while prolonged high temperatures can result in almost total yield loss⁹ (Semenov et al 2011). At vegetative stage, wheat is more vulnerable to high temperatures during the reproductive stage.

The temperature at flowering and grain filling stage vary from 12°C to 22°C on average¹⁰ (Sharma et al., 2019). The high temperature causes a decrease in grain filling duration, which is followed by a loss in grain weight. In wheat, every 1°C increase over 15-20°C reduces grain filling time by 2 to 8 days¹¹ (Xin et al., 2019). Ten days from anthesis until maturity, a high temperature of 35/20°C reduced grain production by 78%, grain number by 63% and individual grain weight by 29%. When it comes to physiological processes, photosynthesis is crucial, and high temperatures have a significant impact. In wheat, the optimal temperature for photosynthesis is a broad range of temperatures (20 to 30°C); however, at temperatures over 30°C, the rate of photosynthesis declines significantly regulating biomass output and grain yield.

Various QTLs have already been reported in previous studies for wheat grain yield¹² (Lopes et al. 2015), Major QTLs for heat tolerance have also been reported on chromosomes 2A and 2B in wheat under heat stress condition. In previous study, wheat have been evaluated in control and heat stress conditions and described as indirect selection criteria for improving grain yield in heat prone environments. Understanding the genetic control of traits associated with grain yield is essential for the enhancing tolerance to heat in plants. It is now recognized that the effective method for genetic improvement of heat adaptive traits in wheat is marker-assisted selection.

In the study by Pinto et al. (2010) common QTL for both drought and heat adaptation were identified on six chromosomes, namely 1B, 2B, 3B, 4A, 4B and 7A¹³. These QTL regions were associated with both agronomic and physiological traits including QTL for grain number, thousand grain weight, canopy temperature, NDVI and chlorophyll content. Likewise, Bennett et al. (2012b) reported two major QTL associated with yield on chromosome 3B¹⁴. The QTL on 3BL explained up to 22% of the phenotypic variation for both yield and canopy temperature. The benefits of a cooler crop canopy, influenced by a combination of leaf, spike, and peduncle cooling were correlated with both yield in the field¹⁵ (Ayeneh et al. 2002) and under controlled conditions¹⁶ (Hede et al. 1999) and associations with differences in plant morphology were also documented¹⁷ (Balota et al. 2008). The association mapping method has been used to wheat, allowing for the finding a large number of quantitative trait loci¹⁸ (Vasantrao et al., 2019). QTL co-localization can also be a powerful way of identifying traits associated with heat tolerance of yield components. These associated traits can give clues about underlying tolerance mechanisms and potentially provide complementary selection criteria for heat tolerance breeding. A breeder needs a handy tool to identify their variety¹⁹ (Pankaj et al. 2021).

In view of this, the present study was designed (1) to identify QTLs with for the physiological and other traits associated with grain yield in the PBW343/IC252874 population (2) to examine the associations of the heat adaptive traits with grain yield in control and terminal heat stress conditions (3) to discover high-yielding and stable DH lines throughout the different environments, three distinct sowing dates, impact of planting dates on wheat yield stability across years.

Materials And Methods

Plant Materials and field trial

By crossing two parental genotypes, PBW343 (heat susceptible) and IC252874 (heat tolerant), a double haploid mapping population (DH) comprised of 165 lines was created. The DHs were developed at Washington State University in Pullman, Washington, and evaluated in the Rajendra Prasad Central Agricultural University's research farm in Pusa, Bihar, India (25° 57' 08" N; 85° 40' 13" E). The generations were advanced using an offseason facility at the Punjab Agricultural University's Research Station in Keylong, Himachal Pradesh, India. During the 2017-2018 and 2018-2019 growing seasons, two trials were conducted (Table 1). In each experiment, three replications of 165 doubled haploid lines along with their parents were sown in an alpha lattice design. The experimental plots were made up of two 2 m long beds with four rows each, spaced 60 cm apart. The agronomic practices recommended for irrigated and normal fertility (125 kg N; 55 kg P₂O₅ and 35 kg K₂O ha⁻¹) conditions were followed throughout the 2 years. Full doses of K₂O and P₂O₅ were applied at the time of sowing. Nitrogen was supplied in the form of split application, 55 kg N ha⁻¹ at sowing, 25 kg N ha⁻¹ at first irrigation (20 days after sowing) and 35 kg N ha⁻¹ at second irrigation (42 days after sowing). Irrigation was provided to the crop at five sensitive growth stages (at crown root initiation Zadok, GS 21; tiller completion Zadok, GS 29; late jointing Zadok, GS 36; flowering Zadok, GS 61; and milk stage Zadok, GS 75) to maintain optimum soil moisture. Weeds were removed manually. The experiment was carried out on three distinct sowing dates, namely November, December, and January, with a gap of twenty-five days between them. In both succeeding crop seasons, the experiment with late sown conditions and very late sown conditions were considered as heat and terminal heat stressed one. To expose the plants at different levels of high temperatures at the time of grain filling, the crop was sown during the second week of each month (Table S1).

Phenotyping

The grain yield (GY in g) was estimated by weighing grains of harvested plants from a 2 m² plot, ignoring boundary impact²⁰ (Sayre et al. 1997). TGW was measured by taking random samples of 500 grains from plot yield and weighed. GFD was estimated as the difference in days between anthesis and physiological maturity. The leaf chlorophyll content was measured with the SPAD unit using a portable chlorophyll metre (SPAD-502 Minolta, Spectrum Technologies Inc., Plainfield, IL, USA) at the grain-filling stage. CT values were taken at three development stages (GS55, GS65, and GS83) on bright sunny days between 1200 and 1400 h, using a hand-held infrared thermometer (Mikron Infrared Inc. NJ, USA) positioned at a 30° angle to the horizontal, 1 m from the plot boundary, and 50 cm above the canopy. Only the middle rows of each plot were employed for data collection to reduce competition with surrounding genotypes for water, light, and other resources required for canopy growth.

Genotyping

The DNA was extracted using coleoptiles (<http://www.triticarte.com.au/content/DNA-preparation.html>). This was done using a consensus map²¹ (Somers et al. 2004). The thermo cycling programme included a 3 minute denaturation at 94°C, 30 second cycles at 50/65°C, 30 second cycles at 72°C, and a final 2

minute cycle at 72°C in Thermal Cycler²² (Sharma et al. 2016). The parents' SSR markers were tested for polymorphism on 322 SSRs. 71 were polymorphic between parental genotypes (Table S2). The 71 SSRs (gwm, wmc, swm, barc, and cfd) were used to build linkage maps across wheat chromosomes. Polymorphic SSR profiles were visually evaluated by coding PBW343 alleles as "A" and IC252874 alleles as "B." "H" stands for heterozygote and 'NA' for missing bands. Allele bands were seen on 3% agarose gels (Fig 1).

Linkage map construction and QTL mapping

The genotyped data of the DH population was used to generate a linkage map using software MapDisto 2.1.7.1. The Kosambi mapping function and interval position type was used for the conversion of recombination frequency into the genetic distance. QTL analysis was performed using QTL Cartographer v2.5²³ (Wang et al., 2010). In the CIM method, forward regression with five background markers, a window size of 10.0 cM, and a walking speed of 2 cM were used in the software Windows QTL Cartographer 2.5. The trait setting for Composite interval mapping (CIM) was done using model 6 and used to determine likely QTL positions and a threshold of 1,000 permutation test at $P = 0.05$. The putative QTLs were defined as two or more linked markers associated with a trait at $LOD > 3.0$. The suggestive QTLs were defined as QTLs where two or more linked markers were detected at $2.0 < LOD < 3.0$ ²⁸ (McIntyre et al. 2010). Here, The LOD value was set as a minimum LOD value 2.0 to keep consider suggestive and minor QTL as well. However, all the QTLs were detected above LOD value 3.0.

Statistical analysis

During both years, analysis of variance (ANOVA) revealed that genotypes differed considerably in heat tolerance for all variables. GenStat-12.1/2009 (<http://www.vsni.co.uk/genstat>) was used to calculate the Pearson correlation coefficient between the attributes in the trials.

G X E analysis

The grain yield data from all the six environments (E1, E2, E3, E4, E5 and E6) were subject to GGE biplot analysis to investigate the impacts of GEI on grain yield. GEAR programme is used to determine the influence of the environment (E), genotype (G), and GE interaction. The first two components acquired from the principle components were utilised by GGE biplot software to create the biplot. The fundamental concept for a GGE biplot is shown below. Where Y_{ij} = the mean yield of genotype i ($i=1,2,...,n$) in environment j ($j=1,2,...,m$), μ is the the grand mean, β_j is the main effect of environment j , ($\mu + \beta_j$) being the mean yield of environment j , λ_l is the singular value (SV) of l th principal component (PC), the square of which is the sum of squares explained by PC_l ($l=1,2,...,k$ with $k \leq \min(m,n)$ and $k=2$ for a two-dimensional biplot), ξ_{il} is the eigenvector of genotype i for PC_l , η_{jl} is the eigenvector of environment j for PC_l , ϵ_{ij} is the residual associated with genotype i in environment j . We need to partition the SVs into the genotype and environment eigenvector in order to generate a biplot which we can used during the visual analysis of MET data due to which the above model can be expressed in the form of Where for for genotype i and environment j the PC_l scores are g_{il} and e_{jl} respectively. The genotype i presented as a

point defined by all g_i values, and environment j is presented as a point defined by all e_j values ($i=1$ and 2 for a two dimensional biplot) in a biplot. If there were the data's that are environment-standardized, to generate the GGE biplot the common formulae would be as follows Where S_j is the standard deviation in environment j , $i=1,2, \dots, k$, g_i and e_j are PC1 scores for genotype i and environment j , respectively²⁴ (Poudel et al., 2020).

Result

Assessment of heat stress

Out of three different dates of sowing, the very late sown experiments were the hottest in terms of average minimum and maximum temperatures during vegetative growth and grain fill, and had the highest number of days with temperature (28 °C, 31°C and 34 °C). The meteorological data for both the cropping season 2017–2018 and, 2018–2019 provided already. An ANOVA of the data collected from 2 years $\times$ 3 environments or dates of sowing $\times$ 5 trait combinations was performed using the software Genstat V. 15. It showed significant ($p < 0.01$) main effects due to genotypes for all traits studied during both the crop seasons 2017-18 and 2018-19 (Table 2). For the five studied traits, values of correlation coefficients based on data pooled over normal, late and very late sowing conditions are presented in (Fig 2).

Phenotypic analyses for heat tolerance

Very contrasting differences were found in parental lines for GFD under stress condition. The heat-tolerant parent, IC 252874, showed non-significant reduction for all yield components, whereas GFD was found highly reduced (22.8% and 24.2%) under late-sown condition in both the years. On the contrary, the heat-sensitive parent, PBW 343, showed significant reduction in GY (41.2% and 33.2%) with little reduction in GFD under late-sown condition in both the years. It was interesting that there was no significant reduction in TGW in both parents under terminal heat stress in very late-sown condition as compared to normal sown. A significant reduction in GFD (17.3% and 19.0%) and GY (33.2% and 30.0%) was observed in DH population under heat stress condition across the years.

QTL analysis for heat stress tolerance

During the crop seasons 2017–18 and 2018–19, composite interval mapping (CIM) discovered a total of twenty one quantitative trait loci (QTLs) over six environments (E1, E2, E3 E4, E5, E6) , with three (NS), seven (LS) and eleven (VLS) conditions. QTLs spanned on linkage groups 1A, 1B, 2A, 2B, 2D, 3B, 4A, 6A, 6B and 7D (Fig 3). The QTLs were detected for the traits viz., grain filling duration (GFD), canopy temperature (CT), chlorophyll (SPAD), thousand grain weight (TGW) and grain yield (GY) respectively. The LOD score of identified QTLs varied from 2.5 (*Qtgw_rpcau_2A*) to 10.4 (*Qspad_rpcau_2D*) during the year, 2017-18 and 2018-19, explaining 5.07% and 15.05% phenotypic variance (PVE) respectively. Most number of QTLs was detected on chromosome 2B and least on 1A, 6A and 6B. The graph below, a representative graph of QTL hit is depicted (Fig 4).

QTL for Morpho-physiological traits

The morpho-physiological traits, canopy temperature (CT) and chlorophyll (SPAD value) was analyzed for QTL mapping. The Prominent QTLs related to CT were found on chromosomes 1B, 2B and 4A. The three QTLs, *Qct_rpcau_2B*, *Qct_rpcau_1B* and *Qct_rpcau_4A* were found in heat stressed environments across the year. Out of the three, one stable QTL (*Qct_rpcau_4A*) in the environment E6 has shown PVE up to 17.85%. On the other hand, four QTLs appeared for SPAD values which were associated with the chromosome 2D, 4A, 6B and 7D. Out of the four QTLs, *Qspad_rpcau_2D*, *Qspad_rpcau_6B* and *Qspad_rpcau_7D* were found in heat stressed environment whereas *Qspad_rpcau_4A* was appeared in normal sown condition. The QTL, *Qspad_rpcau_2D* was found to be stable explaining the PVE of 15.05%.

QTL for grain yield and related attributes

The three yield and related parameters were analyzed for the mapping study viz. GFD, TGW and GY. A total no. of six QTL was identified for the grain filling duration (GFD), four QTLs each thousand grain weight (TGW) and for grain yield (GY). A stable QTL for GFD (*Qgfd_rpcau_2A*) was found on chromosome 2A and accounted for 16.41% of PVE. *Qtgw_rpcau_1B* was identified to be a stable QTL for TGW with the PVE up to 17.54%. For GY, three stable QTLs viz. *Qgy_rpcau_3B*, *Qgy_rpcau_4A* and *Qgy_rpcau_6A* were associated with the chromosomes 3B, 4A and 6B explaining a PVE of 20.85%, 17.43% and 17.37% respectively.

GGE biplot (Which-won-where/model)

For two years, biplot analysis of the double haploid lines revealed a varied response under three distinct sowing dates or environments. For normal sowing, late sowing, and very late sown sowing, biplots were created. The polygon was created by connecting the DH lines that were farther away from the biplot origin, ensuring that all other DH lines were included. Because they were the farthest from the biplot's origin, the DH lines on the polygon's vertices performed either the best or the worst in one or more year or conditions. The GGE biplot's 'polygon' (which-won-where) view splits the biplot into sectors using perpendicular lines that passes through the polygon sides. The polygon is created by combining the biplot's extreme genotypes. If environments are divided into sectors, different genotypes will win in various sectors, resulting in a crossover GE pattern. The vertex genotype is the winning genotype for an environment or combination of environments in a sector. In contrast, if all environments are classified as belonging to the same sector, a single genotype has the best yield across all conditions. In all the environmental conditions, the vertex genotype in a sector with no environment is regarded a poor performer. Within the polygon, genotypes were less sensitive to location than genotypes at the vertex. In this study, feature of the biplot identified winning genotypes for the trait grain yield fell into 9 or 8 sectors for Y1 and Y2 respectively (Fig 5). The vertex or winning DH lines for GY those were found to be common across the sowing dates (D1, D2, D3, D4, D5, D6) and year (Y1 and Y2) are DH 34, 146, 170, 149, 195 and 60. Surprisingly, in virtually all settings, the highest actual and anticipated yielding lines are almost identical. This shows that various DH lines triumphed in different areas, and that there was a crossover GE pattern.

Mean vs. stability model

The GGE biplot's 'mean vs. stability' perspective explained 75-80% of genotypic and genotype environment variance across environments at various sowing dates. The arrow on the AEC abscissa indicates in the direction of genotypes with better yield performance and ranks them according to yield performance. Each genotype's stability was investigated by projecting it onto the AEC vertical axis. The most stable genotype had a near-zero projection onto the AEC abscissa (horizontal axis) and was virtually on the AEC abscissa (horizontal axis) (vertical axis). The DH lines for grain yield, DH 145, 125, 107, 35, 164, 18, 113, 104, found to be most stable in all the dates (D1, D2, D3, D4, D5, D6) across the year (Y1 and Y2) (Fig 6).

Discussion

The experiment was carried out with a 165 doubled haploid population produced from a PBW343 X IC252874 cross. The different planting dates resulted in a total of six different environmental conditions. The mean minimum and maximum temperatures under late sowed and very late sown circumstances were higher than the normal sown condition for the 2017–2018 and 2018–2019 crop seasons. The very late sown condition had the lowest yield on average for the population because to the higher temperature and longer day duration. During the flowering stage, the crop was more sensitive to high temperatures²⁵ (Dolferus et al. 2011). There was considerable variability among the parents of the DH population for the traits studied, reflecting genetic diversity among both the parents and the derived population. For all of the investigated variables present in the DH lines for heat tolerance, analysis of variance indicated significant ($p < 0.01$) main effects associated to genotypes. Values of correlation coefficients based on data aggregated across Normal sown, Late sown, and Very Late Sown circumstances for the investigated morpho-physiological and yield related variables.

We could detect 21 QTLs with additive effect for all studied traits in NS, LS and VLS conditions, respectively. This confirms these traits are genetically complex and also inherited quantitatively as previously reported (Lopes et al. 2015)¹². The results of this study are in agreement with those of reported in previous studies (Azam et al. 2015)²⁶. For example, Pinto et al. (2010) reported eight QTLs for GY¹³.

QTL comparison between conditions

Investigation of a double haploid population in the six (E1, E2, E3, E4, E5 and E6) environmental conditions allowed us to compare detected QTLs for different traits and also to identify common QTLs between the different conditions. In this Study, seven QTLs with common flanking marker were detected between the six environments. The common QTLs were shared on the chromosome 2A, 3B and 4A. The marker, Gwm47-gwm445 flanked the two QTLs, *Qtgw_rpcau_2A* (E1) and *Qgfd_rpcau_2A* (E6) for TGW and GFD, respectively. The marker, Gwm533.2-wmc500 was linked to two QTLs for GY and GFD, *Qgy_rpcau_3B* (E3) and *Qgfd_rpcau_3B* (E6), respectively. *Qgy_rpcau_4A* (E3), *Qspad_rpcau_4A* (E4), and *Qct_rpcau_4A* (E5) were shown to be associated with the marker Cfd71-wmc48. These environment-

specific QTLs can also be employed to improve breeding efficiency using the marker-assisted selection method. The common QTLs influenced GY, WSC, CMS, PRO, and Fv/Fm in both environments and were found on chromosomes 2B, 2D, 4A, 4B, 5B, and 6A. However, numerous QTLs with significant impacts (such as QChl-4A) were found for different traits in just one of the two environmental conditions²⁷ (Hassan et al., 2018). Under heat and drought stress, Pinto et al. 2010 found multiple environment-sensitive QTLs for grain yield and yield-related characteristics in this group¹³.

Co-location and pleiotropy of QTLs

In the present study, the co-location of two or more QTLs confirmed the strong correlations among the studied traits. For example, *Qspad_rpcau_7D* and *Qgfd_rpcau_7D* coincided in their genomic regions with each other and suggested that the strong positive correlation between SPAD values with GFD would come from these co-localized QTLs. Another example of co-location was the QTL near the flanking marker Gwm533.2-wmc500 on chromosome 3B, which affected GY (*Qgy_rpcau_3B*) and GFD (*Qgfd_rpcau_3B*) confirmed their significant positive correlations in the heat stress condition. Previous studies have shown similar co-locations; for example, McIntyre et al. (2010) found multiple co-located QTLs for distinct characteristics in this group; for example, QTLs for WSC and GY were found in the same location on 6D²⁸. On chromosome 7D, Tahmasebi et al. 2016 discovered shared QTLs for GY and SPAD²⁹. A linkage between two genes or the pleiotropic influence of one gene might result in common genomic areas for diverse phenotypes¹³ (Pinto et al. 2010). The findings demonstrated, however, that co-located QTLs with adequate effects for grain yield and yield components in non-stress and heat stress conditions might be beneficial for marker assisted selection or gene cloning to develop high-performance genotypes.

Comparison with previous studies

The results demonstrated that QTLs were mapped for GY trait on chromosomes 2B, 3B, 4A and 6A. Bennett et al. 2012¹⁴ and Hassan et al., 2018²⁷ has reported that GY was associated with 2B. A QTL was detected which influenced the response of grain weight to a brief heat stress applied at early grain filling in a growth chamber, QTL11 (*QHsgw.aww-3B*) with the former having the strongest and most reproducible effect³⁰ (Shirdelmoghanloo et al., 2016). QTL for more than one yield component HSI co-localized in regions 1A, 2A, 2B, and 4A, as well as areas where HSI co-localized with other phenotypic traits (1B, 2A, 2B, 3B, and 5A)³¹ (Mason et al., 2010). However, we have identified chromosome 6A associated with GY (*Qgy_rpcau_6A*) which is not found in previous studies. It explained a PVE of 17.37% in very late sown condition (E6) of the year 2018-19. Therefore it could be a novel QTL for GY and the linked marker Barc206-barc23 could be utilised for MAS. In our study, the QTLs for GFD were harboured on 2A, 2B, 2D, 3B and 7D. Similarly, the QTL responsible for GFD variation was found on chromosome 2D, *QHGfd.bhu1-2D* was flanked by gwm349 and wPt9797 (5.1cM window), with a mean LOD score of 4.5 and a mean percent PVE of 21.0 during the three years, whereas QHGfd.bhu2-2D was flanked by cfd233 and cfd044 (29.8cM window), with a mean percent PVE of 20.6 percent and a mean LOD. On the other hand, we discovered a QTL for GFD on 2D that explains a percent PVE of 13.41 percent on a LOD

value of 7.2³² (Tiwari et al., 2013). In a previous study, two QTLs viz. *QGfd.tam-2A* and *QGfd.tam-2D* were also found on chromosome 2A and 2D. Our finding also does comply with the previous result. However, we found 2B, 3B and 7D associated with trait GFD in our study but not appeared in previous findings. Hence, these loci could be influenced by heat stress in relation to GFD. In our study, the chromosome 1A, 2A, 2B, 2D harboured TGW related QTLs. We have found 4 QTLs for TGW. In heat stressed conditions, a QTL, *Qtgw_rpcau_1B*, flanked by the marker Wmc44-wmc367, accounted for a percent PVE of 17.54 % with a LOD value of 8.9 and found to be a stable QTL for TGW. The QTL (*Qtgw_rpcau_2A*) for TGW was found in normal sown condition with a non significant percent PVE of 5.07% with the LOD value of 2.5. Similarly, Tahmasebi et al., 2016 reported a QTL related to TGW found on chromosomes 2A had no significant QQE effects. Our finding matches with the previous results²⁹. However, in our case, 1A, 2B and 2D were also associated with QTL related to TGW but previous findings have not shown TGW-QTLs on these above chromosomes. Therefore, these chromosomes and linked marker could harbour vital information related to TGW considering high temperature. Other than the yield parameters, morpho-physiological traits like CT and SPAD value were also mapped in relation to heat stress. The CT related QTLs were detected on 1B, 2B and 4A where, *Qct_rpcau_4A* was found to be stable QTL with the percent PVE of 17.85%. Similarly, the QTL for CT was reported on chromosome 2B, with 15% PVE³³ (Paliwal et al., 2012). Tahmasebi et al., 2016 also detected CT related QTL on 1B²⁹. Similarly, Pinto et al. (2010) discovered CT related QTL on 1B as being solely associated with stress environments, with SeriM82 alleles dominating adverse impacts (high CT)¹³. In our study, QTL for SPAD value were identified on 2D, 4A and 7D. *Qspad_rpcau_2D* was detected as a stable QTL for SPAD explaining percent PVE of 15.05% with the LOD value of 10.04 in heat stressed condition. Similarly, the SPAD QTLs were dispersed on 2D and 7D, explained 4.3%–17.3% (PVE) of the total variation in chlorophyll content²⁹ (Tahmasebi et al., 2016). Heat tolerance in field crops, such as bread wheat, is linked to a number of physiological, biochemical, and morphological characteristics²⁷ (Hassan et al., 2018). As a result, we examined a variety of physiological and developmental variables to learn more about the components that influence heat responsiveness to grain weight and the basis of the QTL-controlled tolerance mechanisms. QTLs were discovered for each of the traits investigated in this study, demonstrating that they are genetically complex and quantitatively inherited, as previously stated¹² (Lopes et al. 2015). The findings of this study are in line with those of previous studies²⁶ (Azam et al. 2015). In the near future, the population will be genotyped using modern techniques such as Genotypic by sequencing (GBS) to identify SNP linked to heat stress.

GGE Biplot and Mean Vs. Stability

Based on these findings, we hypothesised that temperatures in the Indo-Gangetic plains climbed somewhat over crop duration but that the pattern remained the same as before. As a result, it's critical to comprehend the unique effects of changed sowing dates and higher temperatures on wheat yield stability in the Indo-Gangetic plains. We discovered differently stable doubled haploid lines for each of these three dates of sowing based on Normal (November), late (December), and very late (January) sown dates. This approach was similarly adopted by Jat et al., 2018 to study stability of diversified wheat

genotypes³⁴. The stability analysis and G X E interaction supported by the QTL analysis data were used to decipher the phenotypic response to normal, late, and very late sown conditions within the population. Performance and stability of genotypes were visualized graphically through the GGE biplot. This can be evaluated by average environment coordination (AEC) method³ (Poudel et al., 2019). The average environment has average PC1 and PC2 scores across environments (Purchase et al., 1997). Greater the absolute length of the projection of a genotype indicates more instability. Furthermore, the average yield of genotypes is approximated by the projections of their markers to the AE Cabsissa³⁶ (Purchase et al., 1997). The greater the absolute length of the projection of a cultivar, the less stable it is. An ideal genotype should have the highest mean performance and absolute stable (less absolute length of projection). The mean yield of DH lines for the different dates of sowing varied across environments (E1, E2, E3, E4, E5, E6) and years (Y1 and Y2) and these results are in agreement with the findings³⁷ (Ahmed and Fayyas-ul-Hassan, 2015). According to Yan and Kang 2003, the polygon perspective of a biplot is the best approach to depict genotype-environment interaction patterns and successfully comprehend a biplot³⁸. The genotypes near the vertices are the most sensitive genotypes, as well as the ones that have shown specialised adaptability³⁹ (Mohammadi et al., 2009). They have a lesser or reduced level of stability. The vertex genotypes were the most sensitive genotypes, according to Yan and Tinker's results, since they are the farthest from the origin in their direction. These genotypes are known as vertex genotypes because they have the greatest distance from the biplot's origin in their directions. The genotypes closest to the origin are the most stable. In this study, feature of the biplot identified winning genotypes for the trait GY fell into 9 or 8 sectors for both the year Y1 and Y2. Similarly, a polygon view of the GGY biplot showed environments divided into 2 or 3 sectors across all management techniques for locations BR, MP, and PB. This suggests that various genotypes triumphed in different areas and that there was a crossover GE pattern³⁴ (Jat et al., 2018). The vertex or winning DH lines for GY those were found to be common across the sowing dates (D1, D2, D3, D4, D5, D6) and year (Y1 and Y2) are DH 34, 146, 170, 149, 195 and 60. The GGE biplot is a useful statistical tool for comparing the performance of genotypes in various contexts. The GGE biplot's polygon view revealed the ideal genotype in each environment. The GGE biplot's "which-won-where" aspect is a useful visual tool for mega-environment study²⁴ (Poudel et al., 2020). The 'average environment coordinate' (AEC) approach of GGE biplot may be referred to as the 'mean vs. stability' view (Yan et al., 2007) since it is based on genotype-focused singular value partitioning (SVP=1)⁴⁰. Genotype comparisons based on mean performance and stability across environments within a mega-environment are made easier with this perspective. Mohammadi et al., 2009 also looked at total crop entries in six different conditions³⁹. The genotypes were further divided into two groups based on the average tester coordinates: high yielding and poor yielding (ATC). The line that passes across the origin demonstrates genotype stability. The vertical black line is a performance line that runs through the biplot's origin and aids in determining a genotype's mean performance, with the right side from the origin exhibiting greater mean grain yield performance. The projections of their markers on the average-tester axis resemble the cultivars' average yield²⁴ (Poudel et al., 2020). The most stable DH lines for GY were DH 145, 125, 107, 35, 164, 18, 113, 104, found to be most stable in all the dates (D1, D2, D3, D4, D5, D6) across the year (Y1 and Y2). Elbasyoni et al., 2018 also

reported similar results in their study⁴¹. Overall, the pattern of DH lines stability varied across the environment and different dates of sowing. To uncover reliable stable lines over different environments, more multi-location trials would be conducted at wheat growing zones. The G X E interaction study might potentially aid in the assessment of heat stress genotypes. This will aid in the pyramiding of DH lines in order to transfer traits directly.

Declarations

The authors declare no competing interest.

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Tables

Table 1: Indication of Year, sowing dates and environments

Year	Sowing Month	Environment	Sowing Condition
Y1 (2017-18)	D1 (November)	ENV1	Normal Sown (NS)
	D2 (December)	ENV2	Late Sown (LS)
	D3 (January)	ENV3	Very Late Sown (VLS)
Y2 (2018-19)	D4 (November)	ENV4	Normal Sown (NS)
	D5 (December)	ENV5	Late Sown (LS)
	D6 (January)	ENV6	Very Late Sown (VLS)

Table 2: Pooled Analysis of variance for different phonological and yield traits

Source of Variation	df	GFD	CT	SPAD	TGW	GY
Env	5	1442.53	1216.21	5.93	61.43	1360.88
Rep	2	8.9	2.38	2.08	4.30	2.06
Block X Rep	48	10.14	4.42	3.48	6.50	9.65
Line	166	14.04	5.69	5.32	4.25	4.02
EnvX Line	830	16.50	2.58	3.54	3.02	5.04

Environment	Trait	QTL Name	Marker interval	LOD score	PVE %
E1(2017-18)	Thousand Grain Weight	<i>Qtgw_rpcau_2A</i>	Gwm47-gwm445	2.5	5.07
E2(2017-18)	Soil plant analysis development	<i>Qspad_rpcau_7D</i>	Barc184-wmc450	6.5	12.18
	Grain Yield	<i>Qgy_rpcau_2B</i>	Gwm614-barc124	4.96	10.83
	Canopy temperature	<i>Qct_rpcau_2B</i>	Wmc25-wmc154	7.43	11.83
E3(2017-18)	Thousand Grain Weight	<i>Qtgw_rpcau_1A</i>	Wmc95-gdm136	4.07	9.74
	Thousand Grain Weight	<i>Qtgw_rpcau_1B</i>	Wmc44-wmc367	8.9	17.54
	Grain Yield	<i>Qgy_rpcau_3B</i>	Gwm533.2-wmc500(C6)	9.39	20.85
	Grain Yield	<i>Qgy_rpcau_4A</i>	Cfd71-wmc48(c7)	6.04	17.43
	Soil plant analysis development	<i>Qspad_rpcau_2D</i>	Wmc470-gwm484	10.4	15.05
	Grain filling duration	<i>Qgfd_rpcau_2B</i>	Wmc213-barc200	6.78	10.64
E4(2018-19)	Soil plant analysis development	<i>Qspad_rpcau_4A</i>	Cfd71-wmc48(c7)	4.4	7.04
	Grain filling duration	<i>Qgfd_rpcau_2D</i>	Gwm484-gwm122	7.1	13.54
E5(2018-19)	Canopy temperature	<i>Qct_rpcau_1B</i>	Wmc719-wmc44	6.4	11.67
	Thousand Grain Weight	<i>Qtgw_rpcau_2D</i>	Wmc112-gwm455	5.9	9.04
	Grain filling duration	<i>Qgfd_rpcau_2B</i>	Barc200-gwm257	5.03	8.28
	Soil plant analysis development	<i>Qspad_rpcau_6B</i>	Gwm191-wmc737	5.3	8.68
E6(2018-19)	Canopy temperature	<i>Qct_rpcau_4A</i>	Cfd71-wmc48	6.04	17.85
	Grain filling duration	<i>Qgfd_rpcau_2A</i>	Gwm47-gwm445	3.43	16.41
	Grain filling duration	<i>Qgfd_rpcau_3B</i>	Gwm533.2-wmc500	7.39	20.85
	Grain filling duration	<i>Qgfd_rpcau_7D</i>	Cfd41-gdm88	7.2	13.41
	Grain Yield	<i>Qgy_rpcau_6A</i>	Barc206-barc23	8.03	17.37

Table 3: Quantitative trait loci detected for grain filling duration, physiological and yield traits in both years 2017–2018 and 2018–2019

Figures

Figure 1

The representative image of wmc500 (SSR marker) showing the marker segregation of 1:1 mendelian ratio in the DH population of PBW 343/IC 252874

Figure 2

Pearson correlations between yield and other traits estimated in trial in the PBW343 X IC252874 DH population.

Figure 3

Linkage map for physiological and yield components in the PBW 343/IC 22874 DHs. QTL detected in both the year 2017-18-19. Markers positions were calculated using the Kosambi mapping function and are listed in cM position from the top of each linkage group.

Figure 4

LOD plots obtained by CIM for the QTL mapping to chromosomes C1-1A, C2-1D, C3-2B, C4-2D, C5-3B, C6-4D, C7-5B, C8-6D. Vertical lines indicate the threshold LOD score for calling a QTL. The chromosomes short arm appears on the top. Single, open-thick triangles indicate the probable position of the centromere.

Figure 5

The polygon (which-won-where) view of genotype main effects plus genotype $\times$ environment interaction effect (GGE) biplot of grain yield of DH lines tested in 2 years, over 3 different dates of sowing.

Figure 6

The mean vs. stability view of main effects plus genotype $\times$ environment interaction effect (GGE) biplot of grain yield of DH lines tested in tested in 2 years, over 3 different dates of sowing.

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