Association analysis for agronomic traits in wheat under terminal heat stress

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ABSTRACT

Terminal heat stress causes irreversible damage to wheat crop productivity. It reduces the vegetative growth and flowering period that consequently declines the efficiency to capture available stem reserves (carbohydrates) in grains. Markers associated with thermotolerant traits ease in marker assisted selection (MAS) for crop improvement. It identifies the genomic regions associated with thermotolerant traits in wheat, but the scarcity of markers is the major hindrance in crop improvement. Therefore, 158 wheat genotypes were subjected to genotyping with 156 simple sequence repeat markers dispersed on three genomes (A, B and D). Allelic frequency and polymorphic information content values were highest on genome A (5.34 (14% greater than the lowest value at genome D) and 0.715 (3% greater than the lowest value at genome D)), chromosome 4 (5.40 (16% greater than the lowest value at chromosome 2) and 0.725 (5% greater than the lowest value at chromosome 6)) and marker xgwm44 (13.0 (84% greater than the lowest value at marker xbarc148) and 0.916 (46% greater than the lowest value at marker xbarc148)).

Bayesian based population structure discriminated the wheat genotypes into seven groups based on genetic similarity indicating their ancestral origin and geographical ecotype. Linkage disequilibrium pattern had highest significant (P < 0.001) linked loci pairs 732 on genome A at r² > 0.5 whereas, 58 on genome B at r² > 0.5. Linkage disequilibrium decay (P < 0.01 and r² > 0.1) had larger LD block (5–10 cM) on genome A. Highly significant MTAs (P < 0.000061) under heat stress conditions were identified for flag biomass (xbarc7) and grain yield (xgwm44) linked loci pairs 732 on genome A at r² > 0.1 whereas, 58 on genome B at r² > 0.5. Linkage disequilibrium decay (P < 0.01 and r² > 0.1) had larger LD block (5–10 cM).

1. Introduction

Wheat is an important staple food crop, grown in a wide range of climatic and soil conditions. It is a source of energy and provides 70–75% calories and 8–15% proteins in a daily diet (Shewry and Hey, 2015). It is not only consumed as human diet, but a large portion of wheat is used to make flour, semolina and raw material for many bakery products. Pakistan is an agriculture-based country and wheat is grown as a staple food in Pakistan (Shaukat et al., 2021; Rizwan et al., 2021; Islam et al., 2021). Rising global warming increases the incidence of disease, changes in the rainfall pattern and overall temperature of the earth (Ahmed, 2020, Ahmed et al., 2020, Ahmad et al., 2019; Ahmed et al., 2019; Ali et al., 2013; Fatima et al., 2020, 2021; Ahmed, 2017; van Ogtrop et al., 2014; Ahmed et al., 2014). It has been reported that mean global temperatures have risen to record 1.2°C higher than previous century (Voozen, 2021). Similarly, work of Schneider et al. (2007) predicted that in 2100 it can go up to 3°C. Determinantal impact of climate change and drought on food and water security have been reported by Ding et al. (2021). Their results suggested that these problems could be solved by using different management options through simulation modeling (Ahmed and Hassan, 2011; Ahmed, 2012; Ahmed et al., 2013, 2014, 2016, 2018; Ahmed and Ahmad, 2012; Ahmed et al., 2016; Ahmed et al., 2017; van Ogtrop et al., 2014; Ahmed et al., 2014).
Protein concentration in wheat crop was significantly affected due to climate change in the work of Asseng et al. (2019), Liu et al. (2019) concluded that under 1.5 °C and 2.0 °C scenarios wheat production will change by −2.3% to 7.0% and −2.4% to 10.5% respectively. Similarly global impact of temperature of <2 °C is not evenly distributed and it will affect regional food security, food prices and trade.

Global temperature has been increased to 1.5 °C since the industrial revolution and it is predicted that increase will be 2.5–4.5 °C till 2100 year due to greenhouse gases emission (Ahmed and Stockle, 2017; Ahmed, 2017). In Pakistan, wheat planted between 15th October to 20th November and each day delay in sowing causes upto 1% yield losses (Moshatati et al., 2017; Ahmed and Farooq, 2013; Akmal et al., 2011). However, 80% of the wheat is late planted due to delay in the physiological maturity of rice and picking of cotton whereas, 20% is planted at normal time in Pakistan (Laghari et al., 2012). Due to late sowing, wheat plant faces terminal heat stress at anthesis and grain filling duration and causes significant yield losses (Aslam et al., 2017). Azmat et al. (2021) suggested early sowing of wheat under changing climate. Furthermore, Gaydon et al. (2021) concluded that improvement in the rice–wheat system water productivity is possible through optimized agronomic changes. Sowing date adjustment is commonly recommended adaptation strategy under changing climate, but it has limitations as reported by Shah et al. (2021).

Abiotic stresses prompted by different climate extreme events could affect crop growth and development. Crops genotypes which can up regulate antioxidant and stress responsive genes could withstand against abiotic stresses (Raja et al., 2020). Mineral nutrition can also be good option to alleviate heat stress in crop plants (Sarwar et al., 2019). Similarly, plant defense mechanisms under stress could be modulated through the application of growth regulators e.g. hydrogen peroxide, salicylic acid, moringa leaf extract and ascobic acid (Sarwar et al., 2018). Brassinosteroids are plant steroid hormones that can induce stress tolerance in plants as reported by Kaur et al. (2018) where they investigated the affects of 28-homobrassinolide seed priming on Brassica juncea seedlings under heat and salinity stress. Melatonin is another good stress defender that can provide physiological protections against environmental stresses (Qi et al., 2018). Heat stress during anthesis damages the reproductive organs associated with spike fertility. High temperatures hinder the microsporogenesis and microgametogenesis which induce spore abortion subsequently reduction in grain formation (Schindfessel et al., 2021; Khan et al., 2021; Talukder et al., 2014). It also inhibits starch accumulation into grains due to granule bound starch, soluble starch and sucrose synthase enzymes activity during grain filling that consequently leads to reduction in grain size, weight and ultimately grain yield (Zahra et al., 2021; Zhao et al., 2008). Grain filling duration and grain filling rate determines grain development. Longer grain filling duration facilitates the longer time to capture available resources and improve the grain weight (Impa et al., 2021; Girousse et al., 2021; Moshatati et al., 2017).

Genetic basis of thermotolerance using genome wide association mapping is useful to improve wheat yield. Recently, mapping and identification of loci controlling thermotolerance traits utilizing microsatellite markers enhances the efficiency of crop improvement faster than conventional breeding techniques (Manjunatha et al., 2021; Mishra et al., 2021; Goel et al., 2019; Hamblin et al., 2011). Plant genomes have large numbers of microsatellite or simple sequence repeats (SSR) that may be dinucleotides, trinucleotides and so on. SSR are PCR-based and co-dominant markers. They are highly polymorphic and discriminate closely related individuals (Sharma et al., 2021; Jones et al., 2009).

Genetic mapping approaches include QTL mapping (traditional linkage mapping) and association mapping (linkage disequilibrium). Quantitative trait loci (QTLs) are the nucleotides sequence on the genome controlling the trait of interest. It requires the progenies/population developed from crosses between desirable parents which includes RILs (recombinant inbred lines), backcross progenies, F2 population, double haploid and near isogenic lines (Kim et al., 2021a, 2021b; Bányai et al., 2021). QTL mapping have advantages viz., rare allele identification, few genetic markers requirement and no population structure effects. Whereas, disadvantages includes long period to develop populations, costly, laborious, limited detection of QTLs and few events of recombination consequently coarse mapping (Kim et al., 2021a, 2021b; Bányai et al., 2021).

Association mapping is alternative to traditional QTL mapping that determine the association between genotypic and phenotypic variation in population. Association mapping requires the presence of linkage disequilibrium that is the non-random association of alleles at different loci (Abou-Ewafa and Shehzad, 2021; Christopher et al., 2021; Kamara et al., 2021; Malik et al., 2021; Jiang et al.2021). Linkage disequilibrium may vary due to different populations, genetic drift, mating system and recombination (Gupta et al., 2005). Understanding the linkage disequilibrium genetic pattern enhances the precision of marker trait association. Strong association between linked loci indicated the presence of linkage disequilibrium decay (Abou-Ewafa and Shehzad, 2021; Christopher et al., 2021; Kamara et al., 2021; Malik et al., 2021; Jiang et al.2021; Stich et al., 2006). Decay of linkage disequilibrium depends on the distance in centimorgon (cM) among alleles. Christopher et al., (2021) and Rafalski, (2002) reported that when decay of linkage disequilibrium is rapid then association mapping resolution would be high.

Marker trait association helps in MAS rather than selection based on phenotypic traits. Nevertheless, limited availability of markers associated with thermotolerant traits prompted this research. Therefore, latest study was designed for the identification of linkage disequilibrium pattern in wheat genotypes to detect association between markers and agronomic traits related to terminal heat stress.

2. Materials and methods

2.1. Plant material and experimental layout

The experiment comprised of 158 wheat genotypes (Supplementary material 1) collected from NARC (National Agricultural Research Center) Islamabad, BARI (Barani Agricultural Research Institute) Chakwal, RARI (Regional Agricultural Research Institute) Bahawalpur, AARI (Ayub Agricultural Research Institute) Faisalabad, Pakistan and CIMMYT (International Maize and Wheat Improvement Center) Mexico viz., 23rd SAWYT (23rd Semi arid wheat yield trial) and 24th SAWYT (24th Semi arid wheat yield trial). Present research work was conducted at the research farm of Pir Mehr Ali Shah Arid Agriculture University Rawalpindi (33.1172°N, 73.0109°E) Pakistan. Genotypes were sown under normal (1st week of November) and heat stress (1st week of December) conditions for three years (2016–2019) in Augmented Complete Block Design with thirteen blocks using check varieties viz., AS-2002 and Aas-11. Twenty plants were selected for phenological data recording at different stages (Zadoks et al., 1974) viz., days to heading (Zadoks scale 55), days to anthesis (Zadoks scale 64), days to maturity (Zadoks scale 88), grain filling duration (Zadoks scale 69–91). Flag leaf area was measured at anthesis stage. Morphological traits that includes plant height, tillers per plant, spike length, spikelet per spike, grains per spike, thousand

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2.2. Genotyping

Deoxiribonucleic acid (DNA) was extracted according to (Randhawa et al., 2009). Briefly, two pieces of one-inch leaves from 14 days old wheat seedlings were placed in each well of plate and lyophilized for 3–4 days. Metallic beads (3 mm, V & P Scientific, USA) were put in each well and shaken in Qiagen Mixer Mill, Model MM-301 (Qiagen, USA) for 6 min at 30 Hz speed. After grinding, 750 μl hot SDS extraction buffer was added and placed on levitation machine (V&Scientific, Inc. USA) for 90 min at 60 °C to obtain 500 μl supernatant. Equal amounts of chloroform:octanol (24:1) were added in each well and centrifuged for 30 min at 4 K rpm. Then 2/3 isopropanol was added and again centrifuged for 30 min at 4 K rpm. Solution was discarded and washed the pallet twice in 70% ethanol. Pallet was dried in air and suspended the pallet in 500 μl TE buffer (500 mM Tris and 50 mM EDTA).

2.3. Marker analysis

A set of 165 simple sequence repeats (SSR) primers were randomly selected covering three genomes and M13 tail (CAGCAGCTTGGAATAACGAC) was synthesized with forward tail primer at 5′ end for polymerase chain reaction with each dye (FAM, HEX, NED or PET). Then 2 μl PCR products (0.5 μl PCR product containing each dye) were diluted in 10 μl loading dye (5 μl formamide and 5 μl DNA ladder) and reaction was performed on DNA analyzer machine ABI-3730 (Scientific, 2014).

2.4. Statistical analysis

Phenotypic data was analyzed by PROC MIXED with block random and entries fixed. BLUP (Best Linear Unbiased Prediction) of mean values were estimated by using statistical software SAS (Scott and Milliken, 1993). Relative performance of recorded data was estimated for agronomic traits following Asana & Williams, (1965). Broad-sense heritability was calculated using the equation:

$$H2 = \frac{\sigma^2G}{\sigma^2G + \sigma^2e/nE}$$

where $\sigma^2G$ is the genotype variance, $\sigma^2e$ represents the variance of the residual and nE is the environments number.

Allelic frequency and PIC (Polymorphism information content) were calculated according to (Liu and Muse, 2005). Population structure was performed on STRUCTURE with burn in length 50,000 cycles and simulation length 100,000 replications. Clusters were assumed 1–12 with 5 independent runs. Evano criteria were utilized to extract DeltaK value in software STRUCTURE HARVESTER v0.6.93 (Evanno et al., 2005). Cluster analysis was performed using Jaccords method with 1000 permutations in software DARwin 6.0 (Perrier and Flori, 2003) and utilizing Unweighted pair-group method with arithmetic mean, dendrogram was constructed by FigTree v1.3.1 (Rambaut, 2009). Principal coordinate analysis was performed using software NTSYS-pc V.2.1 (Rohlf, 2000).

Linkage disequilibrium (LD) was calculated for each paired loci among P-value < 0.001 and allelic frequency correlation ($r^2$) in TASSEL V4.3.1 (Bradbury et al., 2007). Extent of LD was estimated at P < 0.01 with $r^2 > 0.1$ according to Breshegello and Sorrells (2006) and scatter plot was developed among syntenic $r^2$ and genetic distance in software SPSS v16.0. Relative kinship matrix was derived from unlinked markers and Q matrix obtained from Population Structure. Mixed Linear Model (MLM) was used to identify marker trait associations at P < 0.001 through integration of phenotypic and marker data in TASSEL V4.3.1. For stringent threshold, Bonferroni correction was calculated at P < 0.00061.

3. Results

3.1. Phenotypic variation and broad sense heritability

In current study, normal planted wheat faced 25–28 °C temperature at anthesis and 28–32 °C at grain filling that was below the threshold level. Delayed planted wheat exposed to high temperature 28–31 °C at anthesis and 32–36 °C during grain filling duration that influenced the wheat crop productivity (Fig. 1). Delayed planting exposes the wheat plant to heat stress, induces preflowering and reduction in grain filling phase. Results of phenotypic traits are presented in Table 1. Genotypes and treatments were significant for all traits whereas, years were non-significant at P < 0.001 (Supplementary material 2). Mean performance revealed that heat stress overall reduced heading period (20.0%), anthesis (16.3%), maturity (14.1%) and grain filling duration (21.3%). Morphological traits viz., plant height (28.4%), leaf area (51.8%), spike length (18.3%), spikelet per spike (15.8%), tillers per plant (27.4%), grains per spike (19.0%), thousand grain weight (16.0%), biomass per plant (17.7%) and grain yield per plant (34.0%) were also reduced under heat stress. Broad sense heritability ranged from 86% (thousand grain weight) to 99% (days to maturity) under normal conditions whereas 71.5% (flag leaf area) to 97.4% (days to anthesis) under heat stress conditions (Table 1).

3.2. Genetic diversity

Allelic frequency and PIC value is the measures of genetic diversity among genomes and chromosomes. The 165 SSR markers were tested on genome A, B and D (59, 57 and 49) as presented in Table 2. Genome A had highest number of alleles (315 alleles, 2–13 alleles per locus) subsequently genome D (262 alleles, 3–9 alleles per locus) and genome B (236 alleles, 3–10 alleles per locus). Allelic frequency was highest on genome A (5.34) followed by genome B (4.82) and genome D (4.60). Among chromosome, highest allelic frequency (5.40) was calculated highest on chromosome 4 and marker xgwm44 (13 alleles) (Tables 3 and 4).

Genome A showed highest PIC value (0.715) ranged 0.221–0.916 followed by genome B (0.689) ranged 0.280–0.885 and genome D (0.695) ranged 0.399–0.859. Among chromosome, PIC value was highest on chromosome 4 with PIC value 0.725 (0.520–0.916)
subsequently chromosome 7 with PIC value 0.723 (0.475–0.859) and chromosome 3 with PIC value 0.712 (0.516–0.872) respectively. PIC value was highest for marker xgwm666 (0.916), xcdf39 (0.885) and xgwm666 (0.879).

### 3.3. Population structure

Genetic similarity among wheat genotypes was assessed by STRUCTURE and distributed 158 genotypes into seven different groups based on maximum likelihood and DeltaK = 7 (Supplementary material 3). Wheat accessions were assigned in seven groups based on maximum likelihood and DeltaK = 7 (Supplementary structure). Wheat accessions were assigned in seven groups based on maximum likelihood and DeltaK = 7 (Supplementary structure). Wheat accessions were assigned in seven groups based on maximum likelihood and DeltaK = 7 (Supplementary structure). Wheat accessions were assigned in seven groups based on maximum likelihood and DeltaK = 7 (Supplementary structure).

Cluster analysis was performed to compare the pattern of wheat accessions with assignments of population structure. Wheat genotypes were distributed in seven clusters viz., cluster 1 (45 accessions), cluster 2 (21 accessions), cluster 3 (19 accessions), cluster 4 (25 accessions), cluster 5 (14 accessions), cluster 6 (18) and cluster 7 (16 accessions) based on genetic dissimilarity (Fig. 3).

Principle coordinate analysis is an alternate approach to Bayesian approach. First three principal components (PCs) explained 20.9% of the total variation in which PC1 retained 10.7%, PC2 6.10% and PC3 4.10 variation (Fig. 4). It also divided the 158 wheat accessions into seven groups. These results were consistence with genotypes assignment generated by cluster analysis and population structure.

### 3.4. Linkage disequilibrium and linkage disequilibrium decay

Simple sequence repeat markers were used to determine the linkage disequilibrium pattern at whole genome as well as individual genome level. In this study, 165 loci identified 17,834 linked loci pairs and 84,086 unlinked loci pairs on whole genome (Table 5). Highly significant loci (P < 0.001) were calculated 1825 linked loci and 7505 unlinked loci in which 1376 linked locus pairs and 5703 unlinked locus pairs were r² > 0.1 respectively.

Genome A had 9538 linked locus pairs and 37,455 unlinked locus pairs. Highly significant locus pairs (P < 0.001 with r² > 0.1) identified 732 linked locus pairs and 2277 unlinked locus pairs. In genome B, 3928 linked locus pairs and 20,670 unlinked locus pairs were identified. Highly significant loci (P < 0.001 at r² > 0.1) were 455 linked and 1621 unlinked locus pairs. Genome D had 4368 linked locus pairs and 25,961 unlinked locus pairs. Highly significant locus (P < 0.001 with r² > 0.1) were 189 linked loci and 1805 unlinked loci were r² > 0.1. However, linkage disequilibrium decay at genome A had larger LD block within 5–10 cM genetic distance at P < 0.01 with r² > 0.1 that was larger than B (<5 cM) and D (<5 cM) genomes (Fig. 5).

### 3.5. Marker trait association

Marker trait associations (MTAs) identified the regions in wheat genome associated with phenotypic traits. Stable MTAs were observed at P < 0.001 under both normal and heat stress conditions (Table 4). Days to heading was significantly associated with markers xwmc243 (2B) and xwmc737 (5B) whereas, days to anthesis with xcdf219 (5B) under normal conditions. Grain filling duration
was linked with xcf2147 (1A), xwmc121 (7D) and xwmc418 (3B) were linked to grains per spike. Marker xbarc7 (2B) was tightly linked with biomass whereas markers xcf2147 (1A) and xwmc671 (7D) with grain yield under heat stress conditions.

4. Discussion

4.1. Phenotypic traits

Terminal heat stress is an important concern in subtropical, tropical, semi-arid and arid regions of the world that reduces the wheat productivity drastically. Optimum temperature for normal wheat growth at heading is 15–20 °C, anthesis 22–25 °C and grain filling duration is 25–28 °C. But temperature above the threshold level at heading (20 ± 1.6 °C), anthesis (26 ± 1.01 °C) and grain filling duration (30 ± 2.13 °C) negatively affects the wheat crop productivity (Khan et al., 2020). In current study, delayed planted crop faced high temperature at anthesis (28–31 °C) and grain filling duration (32–36 °C) that reduces the wheat productivity drastically. Optimum temperature for normal wheat growth at heading is 15–20 °C, anthesis 22–25 °C and grain filling duration is 25–28 °C (Asseng et al., 2015; Tack et al., 2015).
metabolic activities (Impa et al., 2021; Chaudhry and Sidhu, 2021; Zhu et al., 2021).

High temperature of 4–5 °C above the optimum temperature at booting, heading, anthesis and post anthesis period declines the crop productivity due to fast completion of different growth phases (Nawaz et al., 2013). Photoperiodic (PPD-D1, PPD-A1) and vernalization (VRN1, VRN2) responsive genes determines the plant development at volatile temperatures and limiting various growth phases (Zhu et al., 2021). It enforces the plant to complete its vegetative growth and enter in reproductive stage that consequently reduces the plant height and fertile tillers (Zhu et al., 2021). Heat stress also reduces the flowering period and consequently declines the efficiency to capture available stem reserves (Talukder et al., 2014; Zhu et al., 2021). Furthermore, heat stress at grain filling inactivates the metabolism of starch synthesis and accumulation into grains that determines the grains weight (Khadka et al., 2020a,b; Zhao et al., 2015; Zhu et al., 2021).

4.2. Genetic diversity

Simple sequence repeats markers distinguish the closely related genotypes due to existence of hyper variable regions in genome (Pidigam et al., 2021; Kim et al., 2021a, 2021b). In current study, 165 SSR markers were utilized to observe genetic diversity among 158 wheat genotypes. Overall, 813 alleles (average 4.92 alleles per locus) with PIC value 0.699 (0.221–0.916) on whole-wheat genome that was higher than previous studies utilizing SSR markers. Allelic frequency and PIC value were 5.5 and 0.600 (Zarei Abbasabad et al., 2016), 4.3 and 0.650 (Hao et al., 2011), 4.8 and 0.610 (Dodig et al., 2010) and 4.6 and 0.653 (Liu et al., 2010) were reported respectively. It might be due to diverse panel and polymorphic markers used in current study (Abou-Elwafa and Shehzad, 2021; Sharma et al., 2021). Allelic frequency and PIC value on each locus determine the genetic diversity on each genome or chromosome. Higher the average allelic frequency and PIC value indicates the higher diversity.
genetic diversity. Current study revealed the allelic frequency and PIC values on individual genome as well as each chromosome of wheat (Sharma et al., 2021; Seetharam et al., 2021). However, allele frequency and PIC values were observed from lowest to highest \( D < B < A \) genome that indicated lowest genetic diversity on \( D \) and \( B \) genome of wheat as compared to \( A \) genome in Pakistani post-green revolution varieties and CIMMYT lines.

### 4.3. Population structure

Population structure assigned 158 wheat accessions of Pakistani post-green revolution varieties into seven subgroups. Three methods viz., population structure, cluster analysis and principal coordinate analysis consistently led this grouping. Consistency of grouping using these three methods has also been reported earlier (Ya et al., 2017; Tascioglu et al., 2016). Population structure differentiation is based on relatedness frequency of accessions to each group as hypothesized by STRUCTURE. UPGMA clustering distributed the wheat accession based on their genetic dissimilarity (Khadka et al., 2020a,b) whereas, principal coordinate analysis was based on their genetic distance. Bayesian approach population structure determined seven groups. Prior, two main groups were expected based on their origin viz., Pakistani varieties and CIMMYT lines but there was greater genetic diversity among wheat accessions which led to seven groups. The unexpected population structure (7 groups not 2 groups) results may be due to numerous factors viz., selection of germplasm by breeders, geographical origin and age of varieties that influence the structure of wheat accessions. Cluster analysis and principal coordinate analysis also validate the results by distributing wheat genotypes into seven groups.

The main source of wheat germplasm in Pakistan was brought from CIMMYT starting from 1965 when Semi dwarf Mexi-pak was released for general cultivation (Khan and Tsunoda, 1970). Mostly accessions were introduced from CIMMYT and crossed with local cultivars to develop new varieties in Pakistan. It might be due to sharing of their common parentage viz., PASTOR, SOKOLL, KAUZ and WBLL among CIMMYT lines and Pakistani accessions. For example, Pakistani variety AAS-11, Dharabi-11 and Ihasan-16 sharing common parentage PASTOR with CIMMYT lines viz., G-302, G-305, G-313, G-317, G-318, G-321, G-322, G-324, G-327 and so on (supplementary material 1). These results indicated that Pakistani genotypes used in the present study sharing the common ancestor due to their genetic similarity within the populations of a group. Additionally, population genetic structure is perquisite for marker trait association to reduce false associations. Therefore, mixed linear model (MLM) was used to remove these false associations in which Kinship matrix (difference in genetic relatedness) and

### Table 5

| Genotype | Linked locus pair | Observed | \( P < 0.001 \) (%) | \( r^2 > 0.1 \) (%) | \( r^2 > 0.2 \) (%) | \( r^2 > 0.5 \) (%) |
|----------|-------------------|----------|---------------------|---------------------|---------------------|---------------------|
| Genome A |                   | 9538     | 1028                | 732                 | 236                 | 21                  |
| Genome B |                   | 3928     | 558                 | 455                 | 243                 | 58                  |
| Genome D |                   | 4368     | 239                 | 189                 | 109                 | 31                  |
| Whole genome |           | 17,834   | 1825               | 1376               | 588                 | 110                |
| Genotype | Unlinked locus pair |       | 37,455              | 3273                | 2277                | 736                 | 62                  |
| Genome A |                   | 20,670   | 2001               | 1621                | 981                 | 322                 |
| Genome B |                   | 25,961   | 2231               | 1805               | 1097                | 390                 |
| Whole genome |           | 84,086   | 7505               | 5703               | 2814                | 774                |
population structure (Q matrix) were included in previous studies (Yu and Buckler, 2006; Zhao et al., 2007).

4.4. Linkage disequilibrium and linkage disequilibrium decay

Linkage disequilibrium is a non-random association of alleles at different loci on the chromosomes that determine the resolution of association mapping. Current study indicated the presence of linkage disequilibrium on genome and chromosome level at $P < 0.001$ with $r^2 > 0.1$ that was the prerequisite for association mapping. Higher level of linkage disequilibrium is expected in wheat than other crops due to high rate of inbreeding. Flint-Garcia et al. (2003) suggested higher level of linkage disequilibrium in wheat than cross pollinated crops viz., sorghum and maize. Thus it was necessary to find the linkage disequilibrium blocks that does not split into smaller blocks. It also determines the number of markers required for association mapping (Dadshani et al., 2021).

Linkage disequilibrium decay indicates the recombination rate which determines the association mapping precision (El-Esawi et al., 2018). Various factors viz., genetic drift, population size, admixtures, selection, mutation and non-random mating leads to variation in LD pattern (Vos et al., 2017). Decay of linkage disequilibrium depends on the distance in centimorgan (cM) among alleles. If linkage disequilibrium decays within the shorter genetic distance then association mapping resolution would be high and vice versa (Dadshani et al., 2021; Breseghello and Sorrells, 2006). In current study, linkage disequilibrium decay was larger on genome A (5–10 cM) that was larger than recent study reported by Sukumaran et al. (2015) who found decay of linkage disequilibrium within 5 cM on D genome whereas, about 2 cM on A and B genome with highly significant paired loci ($P < 0.01$) and $r^2 = 0.02$ in wheat whereas, Zarei Abbasabad et al. (2016) demonstrated LD decay up to 40–60 cM at lower $r^2 = 0.05$ in Iranian wheat varieties. This might be due to higher genetic diversity in Pakistani germplasm used in this study.

4.5. Marker trait association

Marker trait association application is promising approach in plant breeding to deal with the limitations faced in linkage mapping (Sharma et al., 2021; Seetharam et al., 2021; Kraakman et al., 2004). It improves the efficiency and precision of indirect selection of thermotolerance traits in breeding programs. In current study we identified significant stable marker trait associations under normal and heat stress conditions at $P < 0.001$. But using stringent threshold level (Bonferroni correction $P < 0.000061$) we could not found any significant MTA under normal conditions whereas, 8 MTAs were observed under heat stress. MTAs for grains per spike were observed on chromosome 1A, 7D and 3B in present study. MTAs for grains per spike were previously also reported on chromosome 1A, 4A, 2B, 3B and 5B under heat stress conditions (Shi et al., 2017). Each significant MTA was identified on 1A, 6A and 2B for flag leaf area, spikelet per spike and biomass per plant respectively whereas, only two MTAs were identified on 1A and 7D under heat stress conditions. MTAs for different agronomic traits on 3A, 4A, 6A, 1B, 2B, 2D and 6D were previously reported normal condi-
tions (Sharma et al., 2021; Seetharam et al., 2021; Gupta et al., 2015; Ain et al., 2015; Zhao et al., 2015) that was different from markers identified and growing conditions in current study. In summary, few markers associated with traits were identified previously on same chromosomes under normal conditions, although heat stress conditions investigated in present study was different from previous studies. It is suggested that significant markers associated with traits under heat stress conditions would be useful in MAS that facilitates the indirect selection of traits rather than selection based on phenotype in further wheat breeding programs against heat stress.

5. Conclusion

Marker trait associations of agronomic traits expedite the efficiency of breeding programs for developing thermotolerant cultivars. Genetic rich regions were identified highest on genome A (A > B > D) in studied wheat genotypes that facilities in targeting this genome to identify more loci related to desired traits. This will also help to enhance the power of genome studies and identification of candidate genes in wheat. Population structure and LD pattern provides the useful information for marker trait association. Population structure, cluster analysis and principal coordinate analysis distributed wheat accessions into seven distinct groups representing genetically diverse germplasm. LD pattern was highly significant (P < 0.001) with high \( r^2 > 0.1 \) value suggested the presence of linkage disequilibrium on wheat genome and chromosomes that was the prerequisite of association mapping. LD decay (5–10 cM) on genome A suggested that this genome requires fewer number of markers to detect target loci related to desirable traits using association mapping than other genomes. Stable significant (Bonferroni correction P < 0.000061) marker trait associations identified under heat stress conditions facilitates the breeding program using MAS and gene pyramiding in wheat.

Table 6

| Trait | Marker | Ch | cM  | P      | R^2  |
|-------|--------|----|-----|--------|------|
| DAH   | xcf219.2 | 5B | 80.2| 0.000327 | 0.0722 |
| DAA   | xwmc243.2 | 2B | 27.0| 0.000821 | 0.0511 |
| DAA   | xwmc737.2 | 6B | 54.0| 0.000316 | 0.0466 |
| GFD   | xcf219.2 | 5B | 80.2| 0.006684 | 0.0655 |
| PM    | xgwm335.3 | 5B | 78.0| 0.000615 | 0.0737 |
| FLA   | xwmc737.4 | 6B | 54.0| 0.000747 | 0.0467 |
| SPS   | xwmc304.3 | 1A | 58.0| 0.000746 | 0.0895 |
| GPS   | xwmc153.3 | 3A | 4.5 | 0.000568 | 0.0926 |
| TGW   | xcf421.1 | 6D | 39.0| 0.000338 | 0.0982 |
| GY    | xwmc28.5 | 5B | 144 | 0.000122 | 0.1057 |
| GY    | xgdm153.5 | 5D | 63  | 0.000897 | 0.0812 |

Heat stress conditions

| Trait | Marker | Ch | cM  | P      | R^2  |
|-------|--------|----|-----|--------|------|
| DAA   | xwmc312.5 | 1A | 76.0| 0.00032 | 0.0497 |
| DAA   | xca2256.3 | 4A | 38.5| 0.00079 | 0.0430 |
| DAA   | xwmc241.2 | 2B | 27.0| 0.00046 | 0.0502 |
| DM    | xwmc473.1 | 4D | 23.0| 0.00046 | 0.0842 |
| PH    | xgwm335.3 | 5B | 78.0| 0.00031 | 0.0791 |
| FLA   | xwmc336.1 | 1A | 34.0| 0.00007 | 0.1158 |
| FLA   | xca2256.2 | 4A | 38.5| 0.00097 | 0.0640 |
| SL    | xwmc278.2 | 1A | 62.0| 0.00096 | 0.0913 |
| SPS   | xwmc553.6 | 6A | 46.0| 0.00004 | 0.1095 |
| SPS   | xbarc142.4 | 5A | 48.0| 0.00076 | 0.0921 |
| SPS   | xbarc54.3 | 6D | 42.0| 0.00024 | 0.1079 |
| GPS   | xwmc11.2 | 3A | 13.0| 0.00052 | 0.0779 |
| GPS   | xca2155.2 | 5A | 153.0| 0.00090 | 0.0562 |
| GPS   | xca2147.4 | 1B | 108.8| 0.00001 | 0.1271 |
| GPS   | xwmc27.1 | 3B | 59.0| 0.00090 | 0.0562 |
| GPS   | xwmc418.2 | 3B | 73.0| 0.00000 | 0.1391 |
| GPS   | xgdm11.1 | 1D | 7.0  | 0.00090 | 0.0562 |
| GPS   | xgdm72.3 | 3D | 34.0| 0.00029 | 0.0845 |
| GPS   | xcf76.3 | 6D | 68.0| 0.00026 | 0.0687 |
| GPS   | xwmc121.3 | 7D | 83.0| 0.00009 | 0.0791 |
| TGW   | xgdm153.5 | 5D | 63.0| 0.00044 | 0.0982 |
| BM    | xca2129.3 | 1A | 79.0| 0.00053 | 0.0745 |
| BM    | xbarc148.2 | 1A | 59.0| 0.00067 | 0.0716 |
| BM    | xbarc7.3 | 2B | 51.0| 0.00002 | 0.1421 |
| BM    | xgwm335.3 | 5B | 78.0| 0.00074 | 0.0704 |
| BM    | xgdm153.5 | 5D | 63.0| 0.00078 | 0.0892 |
| GY    | xwmc11.2 | 1A | 13.0| 0.00018 | 0.1007 |
| GY    | xca2256.2 | 4A | 38.5| 0.00086 | 0.0636 |
| GY    | xbarc197.1 | 5A | 117.0| 0.00029 | 0.0947 |
| GY    | xbarc8.1 | 1B | 25.0| 0.00091 | 0.0629 |
| GY    | xgwm18.1 | 1B | 33.0| 0.00060 | 0.0676 |
| GY    | xca2147.4 | 1B | 108.8| 0.00004 | 0.1201 |
| GY    | xwmc737.2 | 6B | 54.0| 0.00035 | 0.0736 |
| GY    | xgdm72.3 | 3D | 34.0| 0.00028 | 0.0951 |
| GY    | xcf76.1 | 6D | 68.0| 0.00060 | 0.0676 |
| GY    | xwmc671.1 | 7D | 111.0| 0.00009 | 0.1095 |

Chr: Chromosome, P: Probability, R^2: Correlation coefficient, DAH: Days to heading, DAA: Days to anthesis, DM: Days to maturity, GFD: Grain filling duration, PH: Plant height, FLA: Flag leaf area, SL: Spike length, SPS: Spikelets per spike, TP: Tillers per plant, TGW: Thousand grain weight, GPS: Grains per spike, BM: Biomass per plant, GY: Grain yield per plant.
Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors contribution

Author Ma (Munir Ahmad), MA (Mukhtar Ahmed) and AK (Adeel Khan) develop the idea, AK conducted the experiment. Mau, MA, and AK have written the manuscript. Phenotypic data was collected in Pakistan under Mau supervision and genotypic data in USA under KSG. MuA, MA, ZA (Zahid Akram) and KSG (Kulvinder Singh Gill) reviewed and edited the manuscript. MA incorporated all suggestions made by the valuable reviewers.

Appendix A. Supplementary Material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2021.08.050.

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