Meta-QTLs, ortho-MQTLs and candidate genes for thermotolerance in wheat (*Triticum aestivum* L.)

Sourabh Kumar

Chaudhary Charan Singh University

Vivudh Pratap Singh

Chaudhary Charan Singh University

Dinesh Kumar Saini

Punjab Agricultural University

Hemant Sharma

Chaudhary Charan Singh University

Gautam Saripalli

Chaudhary Charan Singh University

Sachin Kumar (✉ sachinkpsingh@gmail.com)

Chaudhary Charan Singh University

Harindra Singh Balyan

Chaudhary Charan Singh University

Pushpendra Kumar Gupta

Chaudhary Charan Singh University

Research Article

Keywords: Wheat, heat stress tolerance, meta-QTLs, ortho-MQTLs, GWAS, candidate genes

Posted Date: October 1st, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-946920/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Version of Record: A version of this preprint was published at Molecular Breeding on November 1st, 2021. See the published version at <https://doi.org/10.1007/s11032-021-01264-7>.

Abstract

Meta-QTL analysis for thermotolerance in wheat was conducted to identify robust meta-QTLs (MQTLs). In this study, 441 QTLs related to 31 heat-responsive traits were projected on the consensus map saturated with 50,310 markers. This exercise resulted in the identification of 85 MQTLs with confidence interval (CI) ranging from 0.11 to 34.9 cM with an average of 5.6 cM. This amounted to a 2.96 fold reduction relative to the mean CI (16.5 cM) of the QTLs used. Seventy-seven (77) of these MQTLs were also verified with the results of recent genome-wide association studies (GWAS). These MQTLs included seven MQTLs that are particularly useful for breeding purposes (we called them Breeders' MQTLs). Seven ortho-MQTLs between wheat and rice genomes were also identified using synteny and collinearity. The MQTLs were used for the identification of 1,704 candidate genes (CGs). *In silico* expression analysis of these CGs permitted identification of 182 differentially expressed genes (DEGs), which included 36 high-confidence candidate genes (CGs) with known functions previously reported to be important for thermotolerance. These high confidence CGs encoded proteins belonging to the following families: protein kinase, WD40 repeat, glycosyltransferase, ribosomal protein, SNARE associated Golgi protein, GDSL lipase/esterase, SANT/Myb domain, K homology domain, etc. Thus, the present study resulted in the identification of MQTLs (including breeders' MQTLs), ortho-MQTLs, and underlying CGs, which could prove useful not only for molecular breeding for the development of thermotolerant wheat cultivars but also for future studies focused on understanding the molecular basis of thermotolerance.

Introduction

Wheat (*Triticum aestivum* L.) is grown in the tropical and sub-tropical regions of the world plagued with various abiotic stresses (Gupta et al. 2020). Heat, drought, floods, salinity, cold and toxic chemicals are all prominent abiotic stresses, which adversely affect crop productivity (Lesk et al. 2016). At the global level, heat and drought are recognized as the two most widely occurring abiotic stresses reducing wheat yield significantly (Gupta et al. 2012, 2020). According to a global climate change model, the average ambient temperature is expected to rise by 6°C by the end of the twenty-first century (De Costa et al. 2011). In India, the temperature during the wheat growing season increased at a rate of 0.04°C per decade during the twentieth century (1901-2000) (Chauhan et al. 2014). A recent estimate suggested that wheat yield can be reduced up to 60% in the field due to extreme heat stress (Zampieri et al. 2017). According to several studies on the effect of rising temperature on wheat grain yield, it is now known that every 1°C rise in average temperature reduces global wheat yield by 6% (Asseng et al. 2011). This rise of 1°C during the grain filling period can cause a yield loss of 0.25 tons ha⁻¹ (~8%, assuming a global average yield of 3,200 Kg ha⁻¹) in winter wheat (Kristensen et al. 2011). In another study, a rise of 2°C was estimated to reduce global wheat yields by 11% (Zaveri and Lobell 2019). Also, based on simulation studies involving agronomic trials conducted from 1985 to 2011, a 1°C rise in mean temperature during reproductive stages could cause as much as a 21% reduction in wheat yield (Barkley et al. 2014).

Studies have also been conducted on the effect of heat stress on physiological traits associated with yield losses. Based on these studies, reduction in wheat yield due to heat stress has been shown to be associated with the following traits: poor seed germination, inactivation of photosynthetic enzymes, reduced photosynthetic capability, slower nutrient transport, lower chlorophyll content, premature leaf senescence, poor pollen viability/germination, shorter grain filling period, fewer and lighter grains, poor starch/protein content resulting into poor bread-making quality (Akter et al. 2017; Bheemanahalli et al. 2019; Jacott and Boden 2020; Jagdish 2020; Djanaguiraman et al. 2020; Poudel and Poudel 2020).

In view of the above, a breeding strategy that enables plants to become resilient to high-temperature stress appears to be an effective and sustainable solution. Therefore, the development of heat-tolerant, high-yielding wheat varieties and/or pre-breeding materials for future breeding programmes is crucial for food and nutritional security. A complete understanding of the genetics of thermotolerance, which depends on a number of interacting QTLs/genes, should certainly help in the development of heat-tolerant cultivars.

Hundreds of QTLs are now known in wheat, which affect various agronomic and quality traits under heat stress (WheatQTLdb by Singh et al. 2021; <http://www.wheatqtladb.net/>). However, limited efforts have been made for the discovery of meta-QTLs (MQTLs) for heat tolerance in wheat (Acuña-Galindo et al. 2015; Liu et al. 2020). BioMercator (Arcade et al. 2004; Sosnowski et al. 2012) and MetaQTL (Veyrieras et al. 2007) are the two most versatile software packages available for MQTL analysis. Algorithms furnished in BioMercator estimate the most appropriate MQTLs using the available information. Meta-QTL analysis may be followed by the selection of promising MQTLs (breeders' QTLs) for marker-assisted breeding (MAB) (Goffinet and Gerber 2000; Yu et al. 2014; Maccaferri et al. 2015) and may also be used for identification of candidate genes (CGs) for future studies.

In the recent past, MQTL analysis in wheat has been carried out successfully to detect robust and stable genomic regions for a variety of traits, including the following: (i) grain yield and associated traits (Gegas et al. 2010; **Bilgrami et al. 2020**; Saini et al. 2021; Yang et al. 2021a), (ii) root morphology traits (**Darzi-Ramandi et al. 2017**; **Soriano and Alvaro 2019**), (iii) **potassium use efficiency** (Safdar et al. 2020), (iv) nutritional and quality traits (Quraishi et al. 2011b; Quraishi et al. 2017; Shariatipour et al. 2021), (v) fusarium head blight resistance (Liu et al. 2009; Mao et al. 2010; Cai et al. 2019; Venske et al. 2019; Zheng et al. 2020), (vi) leaf rust resistance (**Soriano and Royo 2015**; **Aduragbemi and Soriano 2021**), (vii) yellow rust resistance (**Jan et al. 2021**), (viii) pre-harvest sprouting tolerance (**Tyagi and Gupta 2012**; Tai et al. 2021), and (ix) **drought tolerance** (**Kumar et al. 2020**). Two of the studies involving meta-QTL analysis for tolerance against heat stress (Acuña-Galindo et al. 2015; Liu et al. 2020); however gave limited information. In one study, two MQTLs and in the other study, 36 MQTLs for heat tolerance alone were reported (Acuña-Galindo et al. 2015; Liu et al. 2020). Acuña-Galindo et al. (2015) reported 43 MQTLs, which provided tolerance against both heat and drought stresses. **The recent study by Liu et al (2020) reported 59 MQTLs using QTLs associated with grain yield and its major components only, thus leaving out several other important traits such as stay green habit, chlorophyll content, chlorophyll fluorescence, etc. Since a large number of studies involving both interval mapping and GWAS for heat stress tolerance have been reported in wheat during the past decade (see WheatQTLdb by Singh et al. 2021), further MQTL analysis for heat-responsive traits is warranted.**

The present study on MQTL and ortho-MQTL analysis was undertaken using the information on available QTLs for heat tolerance (or thermotolerance) in wheat and the known MQTLs in rice along with information on synteny and collinearity between wheat and rice genomes (Ahn et al. 1993; Kumar et al. 2009). The MQTLs were also verified using available information on GWAS and known genes for thermotolerance in wheat. Important genes including high confidence CGs relevant for thermotolerance in wheat were also identified computationally using the information on MQTLs and the reference sequence of the bread wheat genome (Zhu et al. 2021).

Materials And Methods

Bibliographic search and collection of data on QTLs

A comprehensive search using Google Scholar (<https://scholar.google.com/>) and PubMed (<https://pubmed.ncbi.nlm.nih.gov/>) was made for reports on QTLs associated with thermotolerance in wheat; this information was supplemented by the information available in WheatQTLdb (see Singh et al. 2021;

<http://www.wheatqtl.db.net/>). The following information for each QTL was collected: (i) traits/parameters relevant to thermotolerance, (ii) most closely associated markers, (iii) chromosomal location of associated markers and QTLs, (iv) logarithm of the odds (LOD) value for each QTL, and (v) percent phenotypic variation explained (PVE%) or R^2 value for individual QTLs. All QTLs were treated as single QTLs, even if they were discovered for more than one trait or detected in more than one environments. Some studies reported QTLs separately under normal (control) and heat-stress conditions, however, we considered only QTLs that were identified under heat stress conditions.

Construction of the consensus map

A saturated consensus genetic map (described as Wheat_Consensus_map_2021) was generated using the following integrated genetic maps: (i) the “Wheat, Consensus SSR, 2004” with 1258 marker loci (Somer et al. 2004); (ii) an integrated map for durum wheat with 2525 markers (Marone et al. 2013); (iii) the SNP-based consensus map of tetraploid wheat with 30,144 markers (Maccaferri et al. 2015); (iv) SNP-based genetic map constructed using the “Illumina iSelect 90K SNP Array” (Wang et al. 2014), and (v) individual genetic maps for different mapping populations reported in previous studies on QTL analysis (Online Resources 1 and 2). The R package LPMerge (Endelman and Plomion, 2014) was used for the construction of the Wheat_Consensus_map_2021. This map was used as a reference map for QTL projection and identification of MQTLs.

Projection of QTLs on the consensus map

The projection of QTLs onto the Wheat_Consensus_map_2021 was performed by BioMercator v4.2 (<https://urgi.versailles.inra.fr/Tools/BioMercator-V4>; Arcade et al. 2004) using the QTLProj command. QTLs were projected on the consensus map following the homothetic approach proposed by Chardon et al. (2004). When confidence interval (CI) was not reported in the original studies, the genetic distance between flanking markers was taken as the CI. When the QTL peak position was projected, CI (95%) was calculated for each locus using different population-specific equations (Darvasi and Soller, 1997; Guo et al. 2006; Liu et al. 2009), which included the following: (i) for backcross and F_2 populations: $CI = 530/NR^2$; (ii) for doubled haploid (DH) lines: $CI = 287/NR^2$, and (iii) for recombinant inbred lines (RILs), CI was calculated as $163/NR^2$, where N is the population size and R^2 is the phenotypic variation explained (%) by the QTL.

Meta-analysis of the QTLs

QTL meta-analysis was performed for each wheat chromosome, separately, using algorithms implemented in BioMercator v4.2 (Goffinet and Gerber 2000; Veyrieras et al. 2007). Depending on the number of projected QTLs per chromosome, the following two distinct approaches were applied, (i) when the number of QTLs per chromosome was 10 or less, the methods proposed by Goffinet and Gerber (2000) was applied, and (ii) when the number of QTLs per chromosome was >10, the method proposed by Veyrieras et al. (2007) was utilized. In the first approach, the model with the lowest Akaike Information Criterion (AIC) value was used to select the best QTL model for determining the number of MQTLs on each consensus chromosome; this approach was followed in several earlier meta-QTL studies (Lu et al. 2018; Venske et al. 2019; Liu et al. 2020). In the second approach, the following five parameters were used to ascertain the correct number of MQTL: (i) Akaike Information Criterion (AIC), (ii) AIC correction (AICc), (iii) AIC3 candidate model, (iv) Bayesian Information Criterion (BIC), and (v) Approximate Weight of Evidence Criterion (AWE). The model with the lowest value in at least three of the five parameters was chosen as the best fit (Chardon et al. 2014). In our meta-analysis, the QTLs identified from the best-fit model were considered as the MQTL (Online Resource 2). The number of MQTLs on each wheat chromosome was determined based on the

genetic positions and 95% CI. Detailed information on statistical procedures and algorithms implemented in the BioMercator are available, elsewhere (Arcade et al. 2004; Veyrieras et al. 2007; Sosnowski et al. 2012).

The physical positions for individual MQTLs were obtained using the nucleotide sequences of the markers flanking the MQTLs; these nucleotide sequences were obtained from published literature or retrieved from GrainGenes (<https://wheat.pw.usda.gov/GG3/>) and/or CerealDB (<https://www.cerealsdb.uk.net/cerealgenomics/CerealsDB/indexNEW.php>) databases. The marker sequences were used to BLAST against the bread wheat reference genome (IWGSC RefSeq v1.0). In some cases, the physical positions of SNP markers were directly obtained from the JBrowse wheat genome browser (<https://wheat-urgi.versailles.inra.fr/Tools/Jbrowse>). The genomic region between the flanking markers was treated as the physical position of the corresponding MQTL.

Comparison of MQTLs with known MTAs and genes

MTAs reported in five earlier genome-wide association studies (GWAS; published during 2019-20) on heat stress-responsive traits were available; these were utilized for comparison with MQTLs detected in the present study. The details of population size, traits, SNP markers used and MTAs identified in these studies are given in Online Resource 3. The physical positions of the MTAs obtained from different databases (such as CerealDB and JBrowse wheat genome browser) were compared with the physical coordinates of the MQTL regions on the same chromosome; an individual MTA occurring within a specific MQTL region was considered co-located. In this manner, MQTLs detected in this study were verified by the MTAs reported in at least one of the five GWA studies.

In addition to the above, the previously known heat stress-responsive gene sequences were subjected to BLAST analysis against the IWGSC RefSeq v1.0 and the physical coordinates of known genes were extracted, and compared with the physical positions of the MQTL regions. The MQTLs matching with sequences of heat-responsive genes were treated as coincident.

Identification of genes and their expression analysis

The genes underlying individual MQTL regions were identified in the 1 Mb interval on either side (upstream and downstream) of the peak position of the MQTL (total 2 Mb region; Jan et al. 2021); for this purpose, the **BioMart tool of EnsemblPlants** (<https://plants.ensembl.org/biomart/martview>) was used. *In silico* expression analysis of genes were carried out using transcriptome data available at wheat expression browser (expression and visualization platform) powered by expVIP (<http://www.wheat-expression.com>; Ramírez-González et al. 2018). **For this purpose, the relevant expression dataset including expression data related to heat stress was utilized (Liu et al. 2015). This RNA-seq dataset consisted of differential expression of genes in a heat and drought tolerant wheat cv. TAM107, grown under heat-stressed condition, with leaf samples collected separately at 1 h and 6 h after heat stress treatment (40 °C); seedlings grown in normal growth condition (22 °C, well-watered) were taken as control (Liu et al. 2015).** The data on expression was available as log2 transformed TPM (transcripts per million) values. Only genes showing fold change ($FC \geq 2$ or $FC \leq -2$ TPM values) relative to control were considered as differentially expressed. Gene ontology (GO) analysis was conducted using the BioMart tool and the heat maps of the expression patterns were generated using the Morpheus program (<https://software.broadinstitute.org/morpheus/>).

Identification of ortho-MQTLs

A total of seven wheat MQTLs, each derived from ≥ 10 QTLs, were used for the identification of ortho- or syntenic MQTLs in rice. Following steps were involved: (i) genes detected in the above seven MQTLs were subjected to

BLAST analysis against rice genome database at EnsemblPlants to identify rice orthologues, (ii) The rice orthologues were extracted with their physical positions from the database, (iii) Physical position of genes underlying wheat MQTLs were compared with the orthologous genes underlying rice MQTLs for heat tolerance (Raza et al. 2020), and (iv) MQTLs harbouring similar genes located on known syntenic genomic positions of wheat and rice were considered as ortho-MQTLs.

Results And Discussion

The QTL data used for conducting meta-QTL (MQTL) analysis in the present study were largely similar to those used in two previous studies (Acuna-Galindo et al. 2015; Liu et al. 2020). This also facilitated comparison across three studies and interpretation of results. A detailed comparison with respect to the data used and the result obtained are presented in Online Resource 4. For identification of MQTLs involved in thermotolerance, integration of GWAS (for MQTL verification), heat-responsive genes, genes underlying MQTLs and their expression, and homology comparison with rice (ortho-MQTL) offer an interesting contrast between the present and two earlier studies. It is worth noting that the chromosomes in the consensus map were more densely populated with markers and the QTLs in the present study. In our analysis, Wheat_Consensus_map_2021 map was prepared with 50,310 markers and 441 QTLs were projected for 31 heat-responsive traits, which was higher relative to those in previous two studies (Acuna-Galindo et al. 2015; Liu et al. 2020). Further, out of 85 MQTLs detected in our study, the genetic position of 18 MQTLs matched with the MQTLs reported in above two studies (Online Resource 5), whereas the remaining 67 MQTLs were perhaps novel which were not reported earlier. Interestingly, two of the 18 MQTLs were exclusively associated with thermotolerance, 14 MQTLs were associated either only with drought or heat/drought tolerance. Overall results suggest that ~20% (16/85) of MQTLs involved in thermotolerance were common among the three studies including ours and therefore provide a genetic basis for future studies on abiotic stress tolerance. Above 16 MQTLs regions associated with heat and drought tolerance-responsive traits can be used for future studies involving fine mapping, cloning, functional analysis, and development of markers for marker-assisted selection.

QTLs associated with thermotolerance

Information on QTLs from 31 interval mapping reports (published during 2008-2020) for heat-responsive traits was collected. Only 28 of these studies (25 for hexaploid and 3 for tetraploid wheat) had complete information (marker positions and PVE values) needed for conducting meta-QTL analysis; 826 individual QTLs (752 from hexaploid and 74 from tetraploid wheat) for 154 relevant traits were available from these 28 studies (Online Resources 1 and 2). The populations involved in these 28 studies included 13 RIL populations, 6 DH populations, 5 F₂/backcross populations, and one family-based population (rarely same population was used in more than one study). The population size in different interval mapping studies varied from 64 (Mason et al. 2010) to 420 (Sharma et al. 2017) and the number of QTLs reported in individual studies ranged from 2 (Beecher et al. 2012) to 118 QTLs (Shirdelmoghanloo et al. 2016) (Fig. 1a).

The above 826 individual QTLs for 154 traits were grouped into 31 categories (Online Resource 6). The number of QTLs on individual chromosomes ranged from a minimum of 13 QTLs on chromosome 1D to a maximum of 90 on chromosome 3B (Online Resource 2). Distribution of QTLs on individual homoeologous groups also differed, with group 2 having the maximum of 197 QTLs, and group 6 having a minimum of 66 QTLs. This distribution of QTLs on the seven homoeologous groups may be attributed to higher gene density of stress-related expressed sequence tags (ESTs) on group 2 chromosomes and the lowest gene density on group 6 chromosomes as reported in an earlier study in wheat (Ramalingam et al. 2006). The distribution of QTLs in three sub-genomes

also differed with sub-genome B carrying the maximum of 350 QTLs, followed by sub-genomes A with 282 and sub-genome D with 194 QTLs (Fig. 1b). This distribution of QTLs on homoeologous groups and sub-genomes is also in agreement with previous MQTL studies in wheat for thermotolerance (Acuña-Galindo et al. 2015; Liu et al. 2020) and drought tolerance (Kumar et al. 2020). The 826 QTLs were further categorized based on their LOD values and PVE%. The LOD score of the individual QTLs ranged from 2 to 60 with an average of 6.22 (Fig. 1c). Nearly half (46.7 %) of all collected QTLs had their LOD scores ranging from 2 to 4. The PVE for individual QTLs ranged from 1 to 60.1 % with a mean PVE of 12 %. Approximately 75 % (619/826) QTLs explained PVE up to 15 %, whereas about 5 % (41/826) QTLs exhibited >30 % PVE (Fig. 1c), suggesting the availability of both the major and minor QTLs for heat stress tolerance. The number of QTLs per trait ranged from 2 (spike length; SL) to 164 (bread-making quality; BMQ) with an average of ~26 QTLs per trait (Fig. 1d).

Consensus map of wheat (Wheat_Consensus_map_2021)

The markers on the Wheat_Consensus_map_2021 included RFLPs, AFLPs, SSRs, DArTs, SNPs, and some well-known gene loci, such as *Vrn*, *Ppd*, *Rht*, *Glu*, etc. The Wheat_Consensus_map_2021 contained 50,310 markers with a total genetic length of 12,280.7 cM, giving a density of 4.1 markers/cM (Online Resource 7). The length of the linkage maps for the individual chromosomes ranged from 280.1 cM (4D) to 894.1 cM (4A), and the number of markers mapped on the individual linkage groups varied from 392 on 4D to 4,121 on chromosome 1B (Fig. S1). The marker density for individual chromosomes ranged from 1.4 markers/cM for 4D to 11.1 markers/cM for 2A. Sub-genome A covered a distance of 4102.7 cM with 19518 markers (4.76 markers/cM), sub-genome B covered a distance of 4579.9 cM with 23058 markers (5.03 markers/cM), and sub-genome D covered a distance of 3598.1 cM with 7734 markers (2.15 markers/cM) (Fig. S1). This Wheat_Consensus_map_2021 appears to be a relatively more saturated (dense) consensus map of wheat relative to the consensus maps used by Acuña-Galindo et al. (2015) and Liu et al. (2020) (Online Resource 4). This resulted in the identification of many more MQTLs in the present study.

QTL projection and MQTL analysis

From the 826 individual QTLs, 441 (53.4 %) QTLs for different heat-responsive traits (Online Resource 2) were successfully projected onto the Wheat_Consensus_map_2021; the remaining 385 (46.6%) could not be projected due to the following reasons: (i) use of marker types that were not used in consensus map, (ii) lack of commonly associated markers between consensus and initial maps, and (iii) very long CI. Eighty-five (85) MQTLs were obtained from 411 individual QTL with at least one MQTL on each of the 21 wheat chromosomes (Table 1, Online Resource 8). The remaining 30 QTLs remained singletons and were therefore not used for further analysis. Identification of 85 MQTLs from 441 QTLs amounted to a 4.83-fold (411/85) reduction in the number of genomic regions or QTLs associated with thermotolerance in wheat. Earlier, while conducting a combined MQTL analysis for heat and drought tolerance in wheat, the 8.09-fold reduction was reported by Acuña-Galindo et al. (2015) and 4.43-fold by Liu et al. (2020). A relatively large reduction in the number of QTLs reported by Acuña-Galindo et al. (2015) was not surprising since comparatively many QTLs (associated with both heat and drought tolerance) were involved in their study.

MQTL analysis can efficiently reduce the size of CI of QTLs by accumulating QTLs information from different populations of diverse genetic origins. In the present study, the CI of the MQTLs ranged from 0.11 to 34.9 cM with a mean of 5.6 cM. The average length of CI of MQTLs was reduced 2.95-fold relative to the CIs in original QTLs, which is quite similar to those reported in previous meta-QTL studies (Acuña-Galindo et al. 2015; Liu et al. 2020; Yang et al. 2021) in wheat. There were significant differences in average CIs of MQTLs that mapped on different chromosomes; CI of MQTLs on 6D was reduced 14.6 times and that of 5A was reduced 8.9 times, followed by 8.8 times for 7A and

8.1 times for 7B (Fig. S2). Twenty seven (27; 31.76%) MQTLs had CI <2 cM. **The physical lengths for individual 85 MQTLs also differed and ranged from 0.058 Mb for MQTL7D.5 to 735.48 Mb for MQTL2B.3, with an average of 198.83 Mb (26 of these MQTLs each covered a physical distance of <20 Mb) (Online Resource 8).** Our results in the present study support the finding of an earlier study of Yang et al. (2021) showing that the CI was proportional to the number of QTLs on which the MQTL was based. These results suggest that an increase in the number of QTLs used for meta-QTL analysis could significantly reduce the size of the CI of MQTL relative to those in the QTLs.

The distribution of MQTLs on 21 individual wheat chromosomes ranged from 1 on 6D to 7 on 7A (Fig. 2, Online Resource 8) and this number did not depend on the number QTLs on individual chromosomes utilized for MQTL analysis. For instance, chromosomes 2A, 2D, and 3B each had many more QTLs but relatively fewer MQTLs. **Several MQTLs had a clustered distribution in the genome, with some having overlapping regions. One of these clusters, consisting of 4 MQTLs, was identified on chromosome 1A physically located between 2.54 and 12.09 Mb (1A.1-1A.4). All MQTLs (except 4B.2) on chromosome 4B were physically clustered within a 5.19-255.38 Mb long genomic region. Similarly, five MQTLs (5B.1-5B.5) were found in the 64.74-711.63 Mb genomic region of 5B. Clustered MQTLs were also available on 5D (245.29-498.4 Mb), 6B (16.82-119.97 Mb), and 7D (4.19-44.56 Mb) (Online Resource 8). Such a clustered pattern of distributions of MQTLs was also reported in three recent studies (Jan et al. 2021; Saini et al. 2021; Yang et al. 2021).**

The number of individual QTLs within an individual MQTL ranged from 2 to 22 (**Table 1**) and a large proportion of MQTLs (65 %, 55/85) was each based on >3 initial QTLs derived from different mapping populations. The following six MQTLs comprised ≥ 12 QTLs: MQTL2B.2 (18), MQTL2B.3 (12), MQTL2D.2 (22), MQTL3B.2 (14), MQTL5A.2 (12), and MQTL7B.3 (13). This is in sharp contrast to earlier studies, where a single MQTL associated with thermotolerance was based on no more than 10 QTLs (Acuña-Galindo et al. 2015; Liu et al. 2020). **The MQTLs that are based on QTLs identified using many more populations and several environments may be the most reliable, robust, and stable for wheat breeding.**

The number of traits controlled by an individual MQTL ranged from a solitary trait controlled by several MQTLs to as many as 14 traits controlled by a solitary MQTL (e.g. MQTL2D.1); 76 of the 85 MQTLs were multi-trait MQTL (Table 1), each controlling more than one trait indicating either the occurrence of pleiotropic genes or tight linkage among genes for different traits. It could also be due to the use of related traits as also shown in the case of MQTL analysis for grain yield and related traits (Saini et al. 2021) and root-related traits in wheat (Soriano and Alvaro, 2019).

The LOD score of individual MQTLs ranged from 2.7 to 29.8 with an average of 5.5 and the PVE ranged from 2.5 to 26.5% with a mean of 11.5%. The MQTLs were subjected to further selection for identification of some promising MQTLs that we liked to call breeders' MQTLs, using the following criteria: (i) CI <2 cM, (ii) average PVE >10%, (iii) average LOD >4, and (iv) involvement of at least 7 QTLs within the MQTL. This exercise resulted in the selection of seven promising MQTLs (2A.4, 3B.2, 5B.3, 6D.1, 7A.1, 7B.1, and 7B.3) that were also validated with results of GWAS (see later). Three other MQTLs (viz., 1B.1, 6A.2, and 6B.2) having high PVE (>20%), were not selected as breeders' QTLs, due to their association with large CI or due to the fact these were each based on fewer initial QTLs. The so-called breeders' MQTLs should be subjected to further research including validation, fine mapping, marker development, and cloning.

Verification of MQTLs using MTAs from GWAS

The physical coordinates of MQTLs were also compared with physical positions of 1034 MTAs for the traits associated with thermotolerance reported earlier in GWA studies; this resulted in the identification of MQTLs

matching with as many as 704 MTAs (involving 139 MTAs, each associated with multiple heat-responsive traits within an individual GWAS or across several GWA studies) (Fig. 2, Online Resource 9). As many as 63 MQTLs were verified with MTAs obtained from GWAS involving spring wheat and 67 MQTLs were identified for winter wheat populations, respectively. However, 53 MQTLs were verified using GWAS involving both spring and winter wheats. The MQTL6B.5 matched with 110 MTAs and MQTL6B.1 matched with 103 MTAs. There were other MQTLs (e.g., 2B.2, 2B.3, and 5A.3) each involving 10 or more initial QTLs and matched with more than 60 MTAs (Online Resource 9). Overall, around 90% of the total MQTLs were reliable in the present study since they were verified using GWAS results. These findings are in sharp contrast to recent studies from Aduragbemi and Soriano (2021) and Yang et al. (2021), where only 38.66% and 62.68% MQTLs, respectively were verified with MTAs obtained from GWA studies. This might be due to the availability of a comparatively large number of MTAs for comparison in the present study. These verified MQTLs provide a basis for accurately mining of high confidence CGs associated with thermotolerance in wheat.

MQTLs co-located with known genes for thermotolerance

Comparison of MQTLs with previously discovered genes for thermotolerance in wheat may also enhance the value of the current efforts targeted at the molecular dissection of heat stress-associated genomic regions. Therefore, the association of MQTLs with known thermotolerance genes was also examined. Nine MQTLs were associated with seven of the 11 genes known for thermotolerance in wheat (Table 2 and Online Resource 10). These nine genes included three different classes: (i) Following four HSP/HSF genes were found in the seven MQTLs regions: *TaHSP17* in MQTL3D.1, *TaHSP101* in MQTL3D.1, 3D.3, *TaHSP90* in MQTL7A.2, 7A.3, 7A.4, 7A.5, and *TaHsfA6f* in MQTL5D.4. The HSPs are a large family of chaperones that play a major role in providing thermotolerance (Kumar et al. 2018; Campbell et al. 2001; Vishwakarma et al. 2018; Xue et al. 2015; Hu et al. 2018; Wahid et al. 2007; Khan et al. 2019). (ii) The gene *TaGF14-JM22* (encoding 14-3-3 protein-like) corresponding to MQTL4A.3 is known to be involved in starch biosynthesis. This gene has a negative association with thermotolerance so that its reduced expression enhances thermotolerance (Guo et al. 2018). (iii) MQTL5B.1 region harboured following two important heat stress-responsive genes: *TaFER-5B* encoding for ferritin and *TaPEPKR2* gene encoding for phosphoenolpyruvate carboxylase kinase-related kinase. The *TaFER-5B* is known to provide thermotolerance through scavenging reactive oxygen species (Zang et al. 2017) and *TaPEPKR2* gene has been characterized for thermotolerance and drought tolerance in wheat and *Arabidopsis* (Zang et al. 2018). *These data suggest that the nine MQTLs (Table 2), which correspond to seven known genes for thermotolerance are promising candidates for the improvement of this trait and could be useful in wheat breeding for climate resilience. Additional efforts are needed to identify functional variants of these genes in the targeted MQTL regions.*

Identification of genes, *in-silico* expression and GO analysis

Mining of genes in genomic regions of 82 MQTLs (except 1D.1, 4B.2, 5A.3) allowed identification of 1,704 non-redundant genes (Online Resource 11), which included the following: (i) 55 genes each with three MQTLs (MQTL1A.4, 2A.1, 7D.4) and a solitary gene each with two MQTL (MQTL4B.5 and 4D.4). (ii) 260 genes, for which no description for functions was available, and another 78 genes, which were either uncharacterized or with no known encoded proteins (Online Resource 11). (iii) A large number of genes encoding a variety of proteins products in more than one MQTL region showing redundancy of genes in the wheat genome. These included 102 genes for protein kinases, 55 for F-box-like domains, 43 for cytochrome P450, 38 for proteins with leucine-rich repeats, 37 for zinc finger RING/FYVE/PHD-type, and 27 for pentatricopeptide repeats (Fig. S3).

Functional relevance of the genes/gene families listed above in category (iii) with thermotolerance has also been documented previously (Park et al. 2014; Agarwal et al. 2018; Chen et al. 2018; Pandian et al. 2020; Kumar et al. 2021; Guérin et al. 2021;). Following are some examples, (i) a mitogen-activated protein kinase (MAPK) was recently shown to be associated with oxidative stress tolerance and carbon assimilation in wheat under terminal heat stress (Kumar et al. 2021); (ii) F-box-like proteins are known to play a major role in plant responses to diverse developmental and stress conditions in wheat (Guérin et al. 2021); (iii) Cytochrome P450s are the largest enzyme family involved in O₂ and/or NADPH dependent hydroxylation reactions. The expressions of cytochrome P450 genes have been shown to play a prominent role in the crosstalk between abiotic and biotic stress responses (Pandian et al. 2020); (iv) leucine-rich repeats are known to act as receptors of external signals e.g., abiotic stresses such as heat, salt and osmotic stresses (Park et al. 2014).

Genes were also subjected to an *in silico* expression analysis under 1h and 6h of heat stress using RNA-seq data available in a public database. As many as 182 genes, belonging to 71 MQTLs, exhibited differential expression; these genes were categorized into up-regulating (FC ≥ 2) and down-regulating (FC ≤ -2) genes. From these genes, 92 were differentially expressed at 1 h of heat stress (16 up-regulated and 76 down-regulated), 116 were differentially expressed at 6 h of heat stress (55 up-regulated and 61 down-regulated) and only 31 were differentially expressed at both conditions (10 up-regulated, 15 down-regulated, 6 up-regulated at one time-point and down-regulated at the other). A representative heat map of some important DEGs is shown in Fig. 3. The genes with uncharacterized proteins need to be subjected to further investigations for determining their roles in thermotolerance.

The above 182 DEGs were also analysed for gene ontology (GO) enrichment (Online Resource 11). The most significantly enriched GO terms associated with biological processes belonged to metabolic (12 genes) and cellular processes (6 genes) (Fig. S4). The most significantly enriched GO terms associated with molecular function were for heterocyclic compound binding (25 genes) and catalytic activity (19 genes). In terms of cellular components, the genes were enriched mainly in the cell membrane and its components such as intracellular anatomical structure and cell periphery.

Thirty six (36) high confidence candidate genes (CGs) were also detected (Table 3), which either exhibited differential expressions under heat stress or were known to be important for traits related to thermotolerance. The role of some of the important high confidence CGs in providing thermotolerance can be summarized as follows: (i) UDP-glucosyltransferase gene redirects the metabolic flux from lignin biosynthesis to flavonoid biosynthesis under abiotic stress (heat, drought, and salt stress) and the accumulation of flavonoid glycosides providing protection against abiotic stress leading to improvement in grain size (Dong et al. 2020), (ii) over-expression of a zinc finger transcription factor conferred improved thermotolerance and grain yield, as demonstrated in *Arabidopsis thaliana* (Agarwal et al. 2018), (iii) functional involvement of soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) in abiotic and biotic stresses, as reported in several plant species (Kwon et al. 2020), (iv) over-expression of MYB genes provided improved thermotolerance and drought tolerance; this has been attributed to enhanced levels of cellular abscisic acid in wheat (Zhao et al. 2017), (v) a KH domain-containing putative RNA-binding protein was reported to be crucial for the regulation of heat stress-responsive gene and thermotolerance in *A. thaliana* (Guan et al. 2013), and (vi) a C2 domain protein was reported to play a key role in the improvement of *A. thaliana* thermotolerance, mainly through the enhancement of antioxidant enzyme activity, leading to enhanced expression of genes for response to heat stress response (Ye et al. 2020). While these potential genes are a promising target for future breeding and genetic studies, it is not apparent whether they are the actual regulators

behind the MQTLs, since many other genes occur within the CIs of the MQTLs. Further studies are warranted to validate the role of these genes.

Ortho-MQTLs for wheat and rice

Chromosomal regions carrying MQTLs that are conserved across more than one cereal were also identified. For this purpose, MQTLs for different traits relevant to thermotolerance in the present study in wheat were compared with MQTLs reported in rice (Raza et al. 2020). Ortho-MQTLs could be identified for only seven wheat MQTLs positioned on chromosomes 1B, 2B, 2D, 3B, 7B, and 7D that correspond to rice-MQTLs (rMQTLs) located on rice chromosomes 1, 4, 5, 6, 7, and 8. This confirms the occurrence of wheat MQTLs that are orthologues to rMQTLs. Detailed information related to orthologous MQTLs and underlying genes along with physical positions and functional description is presented in Table 4 and Online Resource 12. As a result of this comparative analysis, two ortho-MQTLs (ortho-MQTL2B.3 and ortho-MQTL2D.1) were considered most promising for revealing gene collinearity within a syntenic region between wheat and rice. For ortho-MQTL2B.3, four underlying CGs on chromosome 2B were found to be syntenous and collinear with similar CGs belonging to rMQTL4.4 on rice chromosome 4 (Fig. 4a and Table 4). The wheat ortho-MQTL2D.1 on chromosome 2D was syntenous with rMQTL7.1 on rice chromosome 7 (Fig. 4b and Table 4). The orthologous genes located at the two most promising syntenic regions (viz., ortho-MQTL2B.3 and 2D.1) were **subjected to further analysis. Ortho-MQTL2B.3 contained at least two heat-responsive genes encoding for protein kinase (Sinha et al. 2011) and amino acid/polyamine transporter (Shen et al. 2016), respectively. Whereas, ortho-MQTL2D.1 included at least 5 heat-responsive genes encoding for the following proteins/products: NAC domain-containing protein (Ren et al. 2021), nucleoside phosphatase (Escobar et al. 2001), PLD-regulated protein1-like (Mishkind et al. 2009), RNA recognition motif domain (Lee et al. 2017), and helicase (Gong et al. 2005).**

The crucial roles of some of these genes associated with Ortho-MQTLs in conferring thermotolerance can be summarized as follows: (i) mitogen-activated protein kinase cascades have been shown to be involved in signalling pathways activated by different abiotic stresses including heat stress in different plant species (Sinha et al. 2011), (ii) polyamine transporter is required for the uptake of extracellular polyamines and is known to play a key role in stabilizing the mRNAs of several important heat stress-responsive genes under heat stress in *Arabidopsis* (Shen et al. 2016), (iii) CRISPR/Cas9 based genome editing and transgenesis demonstrated a transcriptional regulatory network with two NAC genes (*ONAC127* and *ONAC129*) coordinating multiple pathways to modulate grain development and heat stress responses at reproductive stages in rice (Ren et al. 2021), (iv) a mitochondrial nucleoside diphosphate kinase (*mtNDPK*) is known to be involved in heat stress response in pea, possibly as a modulator of a protein (86 kilo-dalton protein) synthesized upon exposure to heat (Escobar et al. 2001), (v) phospholipase D and phosphatidylinositol 4,5-bisphosphate are accumulated at the plasma membrane and nucleus, thereby regulates the ion channels and the cytoskeleton under heat stress in *Arabidopsis* and rice (Mishkind et al. 2009), and (vi) the RNA recognition motif of *Arabidopsis* splicing factor affects the alternative splicing of pre-mRNAs of genes, including *FLOWERING LOCUS M* and a heat shock transcription factor involved in the regulation of flowering and adaptation to heat stress (Lee et al. 2017). Among the remaining five ortho-MQTLs, only one ortholog matched between wheat and rice MQTLs (Table 4).

Ortho-MQTLs in wheat in particular and cereals in general, have been examined sparingly; at least four such examples are available (Quraishi et al. 2011a; Khahani et al. 2020, 2021; Saini et al. 2021). The discovery of ortho-MQTLs in closely related species increases their significance and verifies their stability, as well as the confidence of associated genes (Khahani et al. 2020, 2021). The orthologous gene detected in the present study

may be functionally validated and gene-based functional markers can be developed for their use in cereal breeding programs (Quraishi et al. 2009).

Conclusions

The present study involved identification of MQTLs using information about the known QTLs for thermotolerance. Improved meta-analysis strategy was used leading to the identification of 85 most stable and robust MQTLs; 77 of these MQTLs were confirmed using available results of recent GWA studies. Seven promising MQTLs (breeders' MQTLs) were recommended for use in MAB for improvement of thermotolerance in wheat. Ortho-MQTLs for wheat and rice identified in the present study allowed identification of conserved genomic regions associated with heat-responsive traits in rice. As many as 1,704 genes underlying 82 MQTLs were also identified which included 182 genes with differential expression under heat stress. In addition, 36 high confidence CGs underlying 24 MQTLs with known functions were identified as being reliable for thermotolerance. These genes may be validated using one or more of the available approaches (including transgenesis, gene editing, and gene knockout strategies). The results may also be used for studies involving proteomics and metabolomics. On validation, these genes can be used in breeding programmes for improving thermotolerance and may also be used as a resource for designing research programmes for understanding the mechanism for thermotolerance in wheat.

Declarations

Funding

This research was funded by “Biotechnology Industry Research Assistance Council (BIRAC), Government of India, New Delhi” and “United States Agency for International Development (USAID), USA” (Grant No. BIRAC/TG/USAID/08/2014).

Conflicts of interest

The authors of this manuscript declare no conflicts of interest

Availability of data and material

Relevant data are included in this paper and its associated Supplementary Information (SI)

Code availability

Not applicable

Author's contribution

SK, HSB and PKG planned this study. SoK, VPS, DKS, HS and SGM performed the literature search, retrieved data, developed consensus map, conducted meta-analysis and wrote the first draft of the manuscript. SK, HSB and PKG critically revised and edited the manuscript. All authors have read and agree to the final version of the manuscript.

Ethics approval and consent to participate

Not applicable

Consent to participate

Not applicable.

Consent for publication

Not applicable

Acknowledgments

The present study was supported by Biotechnology Industry Research Assistance Council (BIRAC), Government of India, New Delhi and United States Agency for International Development (USAID), USA. HSB was awarded Honorary Scientist position by Indian National Science Academy (INSA), New Delhi during the course of this study. The Head, Department of Genetics and Plant Breeding, Ch. Charan Singh University, Meerut provided necessary facilities for this study.

References

- Acuña-Galindo MA, Mason RE, Subramanian NK, Hays DB (2015) Meta-analysis of wheat QTL regions associated with adaptation to drought and heat stress. *Crop Sci* 55:477-492
- Aduragbemi A, Soriano JM (2021) Unravelling consensus genomic regions conferring leaf rust resistance in wheat via meta-QTL analysis. *bioRxiv*. doi: <https://doi.org/10.1101/2021.05.11.443557>
- Agarwal P, Khurana P (2018) Characterization of a novel zinc finger transcription factor (*TaZnF*) from wheat conferring thermotolerance in Arabidopsis. *Cell Stress Chaperones* 23:253-267
- Ahn S, Anderson JA, Sorrells ME, Tanksley SD (1993) Homoeologous relationships of rice wheat and maize chromosomes. *Molec Gen Genet* 241:483-490
- Akter N, Rafiqul Islam M (2017) Heat stress effects and management in wheat. A review. *Agron Sustain Dev* 37:37
- Ali MB, Ibrahim AM, Malla S, Rudd J, Hays DB (2013) Family-based QTL mapping of heat stress tolerance in primitive tetraploid wheat (*Triticum turgidum* L.). *Euphytica* 192:189-203
- Arcade A, Labourdette A, Falque M, Mangin B, Chardon F, Charcosset A, Joets J (2004) BioMercator: integrating genetic maps and QTL towards discovery of candidate genes. *Bioinformatics* 20:2324-2326
- Awlachew ZT, Singh R, Kaur S, Bains NS, Chhuneja P (2016) Transfer and mapping of the heat tolerance component traits of *Aegilops speltoides* in tetraploid wheat (*Triticum durum*). *Mol Breeding* 36:1-15
- Azam F, Chang X, Jing R (2015) Mapping QTL for chlorophyll fluorescence kinetics parameters at seedling stage as indicators of heat tolerance in wheat. *Euphytica* 202:245-258
- Barakat M, Al-Doss A, Moustafa K, Motawei M, Alamri M, Mergoum M, Sallam M, Al-Ashkar I (2020) QTL analysis of farinograph and mixograph related traits in spring wheat under heat stress conditions. *Mol Biol Rep* 47:5477-5486
- Barakat MN, Al-Ashkar IM, Al-Doss AA, Moustafa KA, Motawei MI, Alamri MS, Mergoum M, Sallam MS (2020) Identification of genotyping-by-sequencing tags associated with bread-making quality traits in spring wheat under heat stress. *Cereal Res Commun* 48:347-353

- Barakat MN, Al-Doss AA, Moustafa KA, Motawei MI, Alamri MS, Mergoum M, Sallam MS, Al-Ashkar IM (2020) Molecular detection of QTLs for flour quality traits in two doubled haploid populations in spring wheat under heat stress. *Cereal Res Commun* 48:525-532
- Barkley A, Tack J, Nalley LL, Bergtold J, Bowden R, Fritz A (2014) Weather disease and wheat breeding effects on Kansas wheat varietal yields 1985 to 2011. *Agron J* 106:227– 235
- Beecher FW, Mason E, Mondal S, Awika J, Hays D, Ibrahim A (2012) Identification of quantitative trait loci (QTLs) associated with maintenance of wheat (*Triticum aestivum* L.) quality characteristics under heat stress conditions. *Euphytica* 188:361-368
- Bennett D, Izanloo A, Reynolds M, Kuchel H, Langridge P, Schnurbusch T (2012) Genetic dissection of grain yield and physical grain quality in bread wheat (*Triticum aestivum* L.) under water-limited environments. *Theor Appl Genet* 125:255-271
- Bennett D, Reynolds M, Mullan D, Izanloo A, Kuchel H, Langridge P, Schnurbusch T (2012) Detection of two major grain yield QTL in bread wheat (*Triticum aestivum* L.) under heat drought and high yield potential environments. *Theor Appl Genet* 125:1473-1485
- Bheemanahalli R, Sunoj VJ, Saripalli G, Prasad PV, Balyan HS, Gupta PK, Grant N, Gill KS, Krishna Jagdish SV (2019) Quantifying the impact of heat stress on pollen germination seed set and grain filling in spring wheat. *Crop Sci* 59:684-696
- Bhusal N, Sarial AK, Sharma P, Sareen S (2017) Mapping QTLs for grain yield components in wheat under heat stress. *PLoS One* 12:0189594
- Bhusal N, Sharma P, Sareen S, Sarial AK (2018) Mapping QTLs for chlorophyll content and chlorophyll fluorescence in wheat under heat stress. *Biol Plant* 62:721-731
- Bilgrami SS, Ramandi HD, Shariati V, Razavi K, Tavakol E, Fakheri BA, Nezhad NM, Ghaderian, M (2020) Detection of genomic regions associated with tiller number in iranian bread wheat under different water regimes using genome-wide association study. *Sci Rep* 10:1-17
- Cai J, Wang S, Su Z, Li T, Zhang X, Bai G (2019) Meta-analysis of QTL for fusarium head blight resistance in chinese wheat landraces. *The Crop Journal* 7:784-798
- Campbell JL, Natalya Y, Hong-gang ZK, Jorge NS, T-HD H, Henry TN (2001) "Cloning of new members of heat shock protein HSP101 gene family in wheat (*Triticum aestivum* (L.) Moench) inducible by heat dehydration and ABA. *Biochim Biophys Acta* 15172:270-277
- Chardon F, Virlon B, Moreau L, Falque M, Joets J, Decousset L, Murigneux A, Charcosset A (2004) Genetic architecture of flowering time in maize as inferred from quantitative trait loci meta-analysis and synteny conservation with the rice genome. *Genetics* 168:2169-2185
- Chauhan BS, Prabhjyot K, Mahajan G, Randhawa RK, Singh H, Kang MS (2014) Global warming and its possible impact on agriculture in India. *Adv Agron* 123:65-121

- Chen G, Zou Y, Hu J, Ding Y (2018) Genome-wide analysis of the rice PPR gene family and their expression profiles under different stress treatments. *BMC genomics* 19:1-14
- Darvasi A, Soller M (1997) A simple method to calculate resolving power and confidence interval of QTL map location. *Behav Genet* 27:125-132
- Ramandi DH, Shariati JV, Tavakol E, Zarini NH, Bilgrami SS, Razavi K (2017) Detection of consensus genomic regions associated with root architecture of bread wheat on groups 2 and 3 chromosomes using QTL meta-analysis. *Aust J Crop Sci* 11:777-785
- De Costa WAJM (2011) A review of the possible impacts of climate change on forests in the humid tropics. *J Natl Sci Found Sri* 39:281-302
- Djanaguiraman M, Narayanan S, Erdayani E (2020) Effects of high temperature stress during anthesis and grain filling periods on photosynthesis lipids and grain yield in wheat. *BMC Plant Biol* 20:268
- Dong NQ, Sun Y, Guo T, Shi CL, Zhang YM, Kan Y, Xiang YH, Zhang H, Yang YB, Li YC, Zhao HY (2020) UDP-glucosyltransferase regulates grain size and abiotic stress tolerance associated with metabolic flux redirection in rice. *Nat Commun* 11:1-16
- Endelman JB, Plomion C (2014) LPmerge: An R package for merging genetic maps by linear programming. *Bioinformatics* 30:1623-1624
- Escobar GML, Marttila S, Håkansson G, Forsberg J, Knorr C (2001) Heat stress response in pea involves interaction of mitochondrial nucleoside diphosphate kinase with a novel 86-kilodalton protein. *Plant Physiol* 126:69-77
- Gegas VC, Nazari A, Griffiths S, Simmonds J, Fish L, Orford S, Sayers L, Doonan JH, Snape JW (2010) A genetic framework for grain size and shape variation in wheat. *Plant Cell* 22:1046-1056
- Goffinet B, Gerber S (2000) Quantitative trait loci: a meta-analysis. *Genetics* 155:463-473
- Gong Z, Dong CH, Lee H, Zhu J, Xiong L, Gong D, Stevenson B, Zhu JK (2005) A DEAD box RNA helicase is essential for mRNA export and important for development and stress responses in Arabidopsis. *Plant Cell* 17:256-267
- Graziani M, Maccaferri M, Royo C, Salvatorelli F, Tuberosa R (2014) QTL dissection of yield components and morpho-physiological traits in a durum wheat elite population tested in contrasting thermo-pluviometric conditions. *Crop Pasture Sci* 65:80-95
- Guan P, Lu L, Jia L, Kabir MR, Zhang J, Lan T, Zhao Y, Xin M, Hu Z, Yao Y, Ni Z (2018) Global QTL analysis identifies genomic regions on chromosomes 4A and 4B harboring stable loci for yield-related traits across different environments in wheat (*Triticum aestivum* L.). *Front Plant Sci* 9:529
- Guan Q, Wen C, Zeng H, Zhu J (2013) A KH domain-containing putative RNA-binding protein is critical for heat stress-responsive gene regulation and thermo-tolerance in Arabidopsis. *Mol plant* 6:386-395
- Guérin C, Mouzeyar S, Roche J (2021) The landscape of the genomic distribution and the expression of the F-Box genes unveil genome plasticity in hexaploid wheat during grain development and in response to heat and drought stress. *Int J Mol Sci* 22:3111

Guo B, Sleper DA, Lu P, Shannon JG, Nguyen HT, Arelli PR (2006) QTLs associated with resistance to soybean cyst nematode in soybean meta-analysis of QTL locations. *Crop Sci* 46:202-202

Guo J, Dai S, Li H, Liu A, Liu C, Cheng D, Cao X, Chu X, Zhai S, Liu J, Zhao Z (2018) Identification and expression analysis of wheat *TaGF14* genes. *Front Genet* 9:12

Gupta M, Chawla V, Garg P, Yadav N, Munjal R, Sharma B (2015) Genetic analysis of yield and heat stress related traits in wheat (*Triticum aestivum* L.) using microsatellite markers. *J Appl Nat Sci* 7:739-744

Gupta PK, Balyan HS, Kulwal P, Gahlaut V (2012) Phenotyping genetic dissection and breeding for tolerance to drought and heat in common wheat: present status and future prospects. *Plant Breed Rev* 36:82-168

Gupta PK, Balyan HS, Sharma S, Kumar R (2020) Genetics of yield, abiotic stress tolerance and biofortification in wheat (*Triticum aestivum* L.). *Theor Appl Genet* 133:1569-1602

Hassan FSC, Solouki M, Fakheri BA, Nezhad NM, Masoudi B (2018) Mapping QTLs for physiological and biochemical traits related to grain yield under control and terminal heat stress conditions in bread wheat (*Triticum aestivum* L.). *Physiol Mol Biol Plants* 24:1231-1243

Hu XJ, Chen D, Lynne McIntyre C, Fernanda DM, Zhang ZB, Drenth J, Kalaipandian S, Chang H, Xue GP (2018) Heat shock factor C2a serves as a proactive mechanism for heat protection in developing grains in wheat via an ABA-mediated regulatory pathway. *Plant Cell Environ* 41:79-98

Jacott CN, Boden SA (2020) Feeling the heat: developmental and molecular responses of wheat and barley to high ambient temperatures. *J Exp Bot* 71:5740-5751

Jagdish SVK (2020) Heat stress during flowering in cereals—effects and adaptation strategies. *New Phytol* 226:1567-1572

Jamil M, Ali A, Gul A, Ghafoor A, Napar AA, Ibrahim AM, Naveed NH, Yasin NA, Mujeeb KA (2019) Genome-wide association studies of seven agronomic traits under two sowing conditions in bread wheat. *BMC Plant Biol* 19:1-18

Jan I, Saripalli G, Kumar K, Kumar A, Singh R, Batra R, Sharma PK, Balyan HS, Gupta PK (2021) meta-QTL analysis for stripe rust resistance in wheat. *Research Square*. <https://doi.org/10.21203/rs.3.rs-380807/v1>

Khahani B, Tavakol E, Shariati V, Fornara F (2020) Genome wide screening and comparative genome analysis for meta-QTLs ortho-MQTLs and candidate genes controlling yield and yield-related traits in rice. *BMC genomics* 21:1-24

Khahani B, Tavakol E, Shariati V, Rossini L (2021) Meta-QTL and ortho-MQTL analyses identified genomic regions controlling rice yield yield-related traits and root architecture under water deficit conditions. *Sci Rep* 11:1-18

Khan A, Ali M, Khattak AM, Gai WX, Zhang HX, Wei AM, Gong ZH (2019) Heat shock proteins: dynamic biomolecules to counter plant biotic and abiotic stresses. *Int J Mol Sci* 20:5321

Kumar A, Saripalli G, Jan I, Kumar K, Sharma PK, Balyan HS, Gupta PK (2020) Meta-QTL analysis and identification of candidate genes for drought tolerance in bread wheat (*Triticum aestivum* L.). *Physiol Mol Biol Plants* 26:1713-1725

- Kumar RR, Suneha G, Singh K, Kavita D, Gyanendra KR, Bhupinder SS (2018) "Characterization of novel heat-responsive transcription factor (*TaHSFA6e*) gene involved in regulation of heat shock proteins (HSPs) A key member of heat stress-tolerance network of wheat. J Biotechnol 279:1-12
- Kumar S, Kumari J, Bhusal N, Pradhan AK, Budhlakoti N, Mishra DC, Chauhan D, Kumar S, Singh AK, Reynolds M, Singh GP (2020) Genome-wide association study reveals genomic regions associated with ten agronomical traits in wheat under late-sown conditions. Front Plant Sci 11:1420
- Kumar S, Mohan A, Balyan HS, Gupta PK (2009) Orthology between genomes of brachypodium wheat and rice. BMC Res Notes 2:1-9
- Kumar U, Joshi AK, Kumari M, Paliwal R, Kumar S, Röder MS (2010) Identification of QTLs for stay green trait in wheat (*Triticum aestivum* L.) in the 'Chirya 3'×'Sonalika'population. Euphytica 1743:437-445
- Kristensen K, Schelde K, Olesen JE (2011) Winter wheat yield response to climate variability in Denmark. J Agric Sci 149:33-47
- Kwon C, Lee JH, Yun HS (2020) SNAREs in plant biotic and abiotic stress responses. Mol Cells 43:501
- Lee KC, Jang YH, Kim SK, Park HY, Thu MP, Lee JH, Kim JK (2017) RRM domain of Arabidopsis splicing factor SF1 is important for pre-mRNA splicing of a specific set of genes. Plant Cell Rep 36:1083-1095
- Lesk C, Rowhani P, Ramankutty N (2016) Influence of extreme weather disasters on global crop production. Nature 529:84-87
- Li L, Mao X, Wang J, Chang X, Reynolds M, Jing R (2019) Genetic dissection of drought and heat-responsive agronomic traits in wheat. Plant Cell Environ 42:2540-2553
- Liu C, Sukumaran S, Claverie E, Sansaloni C, Dreisigacker S, Reynolds M (2019) Genetic dissection of heat and drought stress QTLs in phenology-controlled synthetic-derived recombinant inbred lines in spring wheat. Mol Breeding 39:1-18
- Liu H, Mullan D, Zhang C, Zhao S, Li X, Zhang A, Lu Z, Wang Y, Yan G (2020) Major genomic regions responsible for wheat yield and its components as revealed by meta-QTL and genotype–phenotype association analyses. Planta 252:1-22
- Liu S, Hall MD, Griffey CA, Mckendry AL (2009) Meta-analysis of QTL associated with fusarium head blight resistance in wheat. Crop Sci 49:1955-1968
- Liu Z, Xin M, Qin J, Peng H, Ni Z, Yao Y, Sun Q (2015) Temporal transcriptome profiling reveals expression partitioning of homeologous genes contributing to heat and drought acclimation in wheat (*Triticum aestivum* L.). BMC Plant Biol 15:1-20
- Lu Q, Liu H, Hong Y, Li H, Liu H, Li X, Wen S, Zhou G, Li S, Chen X, Liang X (2018) Consensus map integration and QTL meta-analysis narrowed a locus for yield traits to 0.7 cM and refined a region for late leaf spot resistance traits to 0.38 cM on linkage group A05 in peanut (*Arachis hypogaea* L.). BMC Genomics 19:887
- Maccaferri M, Ricci A, Salvi S, Milner SG, Noli E, Martelli PL, Casadio R, Akhunov E, Scalabrin S, Vendramin V, Ammar K (2015) A high-density SNP-based consensus map of tetraploid wheat as a bridge to integrate durum and bread

wheat genomics and breeding. *Plant Biotechnol J* 13:648-663

Mao SL, Wei YM, Cao W, Lan XJ, Yu M, Chen ZM, Chen GY, Zheng YL (2010) Confirmation of the relationship between plant height and Fusarium head blight resistance in wheat (*Triticum aestivum* L.) by QTL meta-analysis. *Euphytica* 174:343-356

Marone D, Russo MA, Laidò G, Vita PD, Papa R, Blanco A, Gadaleta A, Rubiales D, Mastrangelo AM (2013) Genetic basis of qualitative and quantitative resistance to powdery mildew in wheat: from consensus regions to candidate genes. *BMC Genomics* 14:562

Mason RE, Hays DB, Mondal S, Ibrahim AM, Basnet BR (2013) QTL for yield, yield components and canopy temperature depression in wheat under late sown field conditions. *Euphytica* 194:243-259

Mason RE, Mondal S, Beecher FW, Hays DB (2011) Genetic loci linking improved heat tolerance in wheat (*Triticum aestivum* L.) to lower leaf and spike temperatures under controlled conditions. *Euphytica* 180:181-194

Mason RE, Mondal S, Beecher FW, Pacheco A, Jampala B, Ibrahim AM, Hays DB (2010) QTL associated with heat susceptibility index in wheat (*Triticum aestivum* L.) under short-term reproductive stage heat stress. *Euphytica* 174:423-436

Mishkind M, Vermeer JE, Darwish E, Munnik T (2009) Heat stress activates phospholipase D and triggers PIP2 accumulation at the plasma membrane and nucleus. *Plant J* 60:10-21

Mohammadi V, Zali AA, Bihamta MR (2008) Mapping QTLs for heat tolerance in wheat. *J Agric Sci Technol* 261-267

Mondal S, Mason RE, Huggins T, Hays DB (2015) QTL on wheat (*Triticum aestivum* L.) chromosomes 1B 3D and 5A are associated with constitutive production of leaf cuticular wax and may contribute to lower leaf temperatures under heat stress. *Euphytica* 201:123-130

Naruoka Y, Sherman JD, Lanning SP, Blake NK, Martin JM, Talbert LE (2012) Genetic analysis of green leaf duration in spring wheat. *Crop Sci* 52:99-109

Paliwal R, Röder MS, Kumar U, Srivastava JP, Joshi AK (2012) QTL mapping of terminal heat tolerance in hexaploid wheat (*Triticum aestivum* L.). *Theor Appl Genet* 125:561-575

Pandian BA, Sathishraj R, Djanaguiraman M, Prasad PV, Jugulam M (2020) Role of cytochrome P450 enzymes in plant stress response. *Antioxidants* 9:454

Park S, Moon JC, Park YC, Kim JH, Kim DS, Jang CS (2014) Molecular dissection of the response of a rice leucine-rich repeat receptor-like kinase (LRR-RLK) gene to abiotic stresses. *J Plant Physiol* 171:1645-1653

Pinto RS, Lopes MS, Collins NC, Reynolds MP (2016) Modelling and genetic dissection of stay green under heat stress. *Theor Appl Genet* 129:2055-2074

Poudel PB, Poudel MR (2020) Heat stress effects and tolerance in wheat: A review. *J Biol Today's World* 9:217

Quraishi UM, Abrouk M, Bolot S, Pont C, Throude M, Guilhot N, Confolent C, Bortolini F, Praud S, Murigneux A, Charmet G (2009) Genomics in cereals: from genome-wide conserved orthologous set (COS) sequences to candidate genes for trait dissection. *Funct Integr Genomics* 9:473-484

- Quraishi UM, Murat F, Abrouk M, Pont C, Confolent C, Oury FX, Ward J, Boros D, Gebruers K, Delcour JA, Courtin CM (2011) Combined meta-genomics analyses unravel candidate genes for the grain dietary fiber content in bread wheat (*Triticum aestivum* L.). *Funct Integr Genomics* 11:71-83
- Quraishi UM, Pont C, Ain QU, Flores R, Burlot L, Alaux M, Quesneville H, Salse J (2017) Combined genomic and genetic data integration of major agronomical traits in bread wheat (*Triticum aestivum* L.). *Front Plant Sci* 8:1843
- Ramalingam J, Pathan MS, Feril O, Miftahudin M, Ross K, Ma XF, Mahmoud AA, Layton J, Rodriguez MA, Chikmawati T, Valliyoda B (2006) Structural and functional analyses of the wheat genomes based on expressed sequence tags (ESTs) related to abiotic stresses. *Genomics* 49:1324-1340
- Ramírez-González RH, Borrill P, Lang D, Harrington SA, Brinton J, Venturini L, Davey M, Jacobs J, Van Ex F, Pasha A, Khedikar Y et al (2018) The transcriptional landscape of polyploid wheat. *Science* 361:6403
- Raza Q, Riaz A, Bashir K, Sabar M (2020) Reproductive tissues-specific meta-QTLs and candidate genes for development of heat-tolerant rice cultivars. *Plant Mol Biol* 104:97-112
- Ren Y, Huang Z, Jiang H, Wang Z, Wu F, Xiong Y, Yao J (2021) A heat stress responsive NAC transcription factor heterodimer plays key roles in rice grain filling. *J Exp Bot* 72:2947-2964
- Safdar LB, Andleeb T, Latif S, Umer MJ, Tang M, Li X, Liu S, Quraishi UM (2020) Genome-wide association study and QTL meta-analysis identified novel genomic loci controlling potassium use efficiency and agronomic traits in bread wheat. *Front Plant Sci* 11:70
- Saini DK, Srivastava P, Pal N, Gupta PK (2021) Meta-QTLs Ortho-Meta-QTLs and candidate genes for grain yield and associated traits in wheat (*Triticum aestivum* L.). *Research Square*. <https://doi.org/10.21203/rs.3.rs-430452/v1>
- Schmidt J, Tricker PJ, Eckermann P, Kalambettu P, Garcia M, Fleury D (2020) Novel alleles for combined drought and heat stress tolerance in wheat. *Front Plant Sci* 10:1800
- Senthil A, Foster I, Turner NC (2011) The impact of temperature variability on wheat yields. *Global Change Biol* 17:997-1012
- Shariatipour N, Heidari B, Richards CM (2021) Meta-analysis of QTLome for grain zinc and iron contents in wheat (*Triticum aestivum* L.). *Euphytica* 217:1-14
- Sharma D, Singh R, Rane J, Gupta VK, Mamrutha HM, Tiwari R (2016) Mapping quantitative trait loci associated with grain filling duration and grain number under terminal heat stress in bread wheat (*Triticum aestivum* L.). *Plant Breed* 135:538-545
- Sharma DK, Torp AM, Rosenqvist E, Ottosen CO, Andersen SB (2017) QTLs and potential candidate genes for heat stress tolerance identified from the mapping populations specifically segregating for Fv/Fm in wheat. *Front Plant Sci* 8:1668
- Shen Y, Ruan Q, Chai H, Yuan Y, Yang W, Chen J, Xin Z, Shi H (2016) The arabidopsis polyamine transporter LHR1/PUT3 modulates heat responsive gene expression by enhancing mRNA stability. *Plant J* 88:1006-1021
- Shiferaw B, Smale M, Braun HJ, Duveiller E, Reynolds M, Muricho G (2013) Crops that feed the world 10 past successes and future challenges to the role played by wheat in global food security. *Food Secur* 5:291-317

- Shirdelmoghanloo H, Taylor JD, Lohraseb I, Rabie H, Brien C, Timmins A, Martin P, Mather DE, Emebiri L, Collins NC (2016) A QTL on the short arm of wheat (*Triticum aestivum* L.) chromosome 3B affects the stability of grain weight in plants exposed to a brief heat shock early in grain filling. *BMC Plant Biol* 16:1-15
- Singh K, Batra R, Sharma S, Saripalli G, Gautam T, Singh R, Pal S, Malik P, Kumar M, Jan I, Singh S et al (2021) WheatQTLdb: a QTL database for wheat. *Mol Genet Genomics* 296:1051-1056
- Sinha AK, Jaggi M, Raghuram B, Tuteja N (2011) Mitogen-activated protein kinase signaling in plants under abiotic stress. *Plant Signal Behav* 6:196-203
- Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). *Theor Appl Genet* 109:1105-1114
- Soriano JM, Alvaro F (2019) Discovering consensus genomic regions in wheat for root-related traits by QTL meta-analysis. *Sci Rep* 9:1-14
- Soriano JM, Royo C (2015) Dissecting the genetic architecture of leaf rust resistance in wheat by QTL meta-analysis. *Phytopathology* 105:1585-1593
- Sosnowski O, Charcosset A, Joets J (2012) BioMercator V3: an upgrade of genetic map compilation and quantitative trait loci meta-analysis algorithms. *Bioinformatics* 28:2082-2083
- Sunil H, Upadhyay D, Gajghate R, Shashikumara P, Chouhan D, Singh S, Sunilkumar VP, Manu B, Sinha N, Singh S, Jain N (2020) QTL mapping for heat tolerance related traits using backcross inbred lines in wheat (*Triticum aestivum* L.). *Indian J Genet* 80:242-249
- Talukder SK, Babar MA, Vijayalakshmi K, Poland J, Prasad PVV, Bowden R, Fritz A (2014) Mapping QTL for the traits associated with heat tolerance in wheat (*Triticum aestivum* L.). *BMC Genet* 15:1-13
- Tesfaye W, Blalock G, Tirivayi N (2021) Climate-smart innovations and rural poverty in ethiopia: Exploring impacts and pathways. *Am J Agric Econ* 103:878-899
- Tiwari C, Wallwork H, Kumar U, Dhari R, Arun B, Mishra VK, Reynolds MP, Joshi AK (2013) Molecular mapping of high temperature tolerance in bread wheat adapted to the Eastern Gangetic Plain region of India. *Field Crops Res* 154:201-210
- Tai L, Wang HJ, Xu XJ, Sun WH, Ju L, Liu WT, Chen KM (2021) Cereal pre-harvest sprouting: A global agricultural disaster regulated by complex genetic and biochemical mechanisms. *J Exp Bot* 72:2857–2876
- Tyagi S, Mir RR, Balyan HS, Gupta, PK (2015) Interval mapping and meta-QTL analysis of grain traits in common wheat (*Triticum aestivum* L.). *Euphytica* 201:367-380
- Venske E, Santos RSD, Farias DDR, Rother V, Maia LCD, Pegoraro C, Oliveira ACD (2019) Meta-analysis of the QTLome of fusarium head blight resistance in bread wheat: Refining the current puzzle. *Front Plant Sci* 10:727
- Veyrieras JB, Goffinet B, Charcosset A (2007) MetaQTL: a package of new computational methods for the meta-analysis of QTL mapping experiments. *BMC bioinformatics* 8:1-16

- Vijayalakshmi K, Fritz AK, Paulsen GM, Bai G, Pandravada S, Gill BS (2010) Modeling and mapping QTL for senescence-related traits in winter wheat under high temperature. *Mol Breed* 26:163-175
- Vishwakarma H, Junaid A, Manjhi J, Singh GP, Gaikwad K, Padaria JC (2018) Heat stress transcripts differential expression and profiling of heat stress tolerant gene *TaHsp90* in Indian wheat (*Triticum aestivum* L.) cv C306. *PloS one* 13:0198293
- Wahid A, Gelani S, Ashraf M, Foolad MR (2007) Heat tolerance in plants: an overview. *Environ Exp Bot* 61:199-223
- Wang S, Wong D, Forrest K, Allen A, Chao S, Huang BE, Maccaferri M, Salvi S, Milner SG, Cattivelli L, Mastrangelo AM (2014) Characterization of polyploid wheat genomic diversity using a high density 90 000 single nucleotide polymorphism array. *Plant Biotechnol J* 12:787-796
- Wang X, Guan P, Xin M, Wang Y, Chen X, Zhao A, Liu M, Li H, Zhang M, Lu L, Zhang J (2021) Genome-wide association study identifies QTL for thousand grain weight in winter wheat under normal-and late-sown stressed environments. *Theor Appl Genet* 134:143-157
- Xue GP, Drenth J, McIntyre CL (2015) TaHsfA6f is a transcriptional activator that regulates a suite of heat stress protection genes in wheat (*Triticum aestivum* L.) including previously unknown Hsf targets. *J Exp Bot* 66:1025-1039
- Yadav OP, Singh DV, Dhillon BS, Mohapatra T (2019) India's evergreen revolution in cereals. *Curr Sci* 116:1805-1808
- Yang Y, Amo A, Wei D, Chai Y, Zheng J, Qiao P, Cui C, Lu S, Chen L, Hu YG (2021) Large-scale integration of meta-QTL and genome-wide association study discovers the genomic regions and candidate genes for yield and yield-related traits in bread wheat. *Theor Appl Genet* 1-27
- Ye Q, Yu J, Zhang Z, Hou L, Liu X (2020) VvBAP1 a grape C2 domain protein plays a positive regulatory role under heat stress. *Front Plant Sci* 11
- Yu Q, Li L, Luo Q, Eamus D, Xu S, Chen C (2014) Year patterns of climate impact on wheat yields. *Int J Climatol* 34:518-528
- Zampieri M, Ceglar A, Dentener F, Toreti A (2017). Wheat yield loss attributable to heat waves drought and water excess at the global national and subnational scales. *Environ Res Lett* 12:6
- Zang X, Geng X, He K, Wang F, Tian X, Xin M, Yao Y, Hu Z, Ni Z, Sun Q, Peng H (2018) Overexpression of the wheat (*Triticum aestivum* L.) *TaPEPKR2* gene enhances heat and dehydration tolerance in both wheat and *Arabidopsis*. *Front Plant Sci* 9:1710
- Zang X, Geng X, Wang F, Liu Z, Zhang L, Zhao Y, Tian X, Ni Z, Yao Y, Xin M, Hu Z, Sun Q, Peng H (2017) Overexpression of wheat ferritin gene *TaFER-5B* enhances tolerance to heat stress and other abiotic stresses associated with the ROS scavenging. *BMC Plant Biol* 17:1-13
- Zaveri E, Lobell DB (2019) The role of irrigation in changing wheat yields and heat sensitivity in India. *Nat Commun* 10:1-7
- Zhao Y, Du H, Wang Y, Wang H, Yang S, Li C, Chen N, Yang H, Zhang Y, Zhu Y, Yang L, Hu X (2021) The calcium-dependent protein kinase ZmCDPK7 functions in heat-stress tolerance in maize. *J Integr Plant Biol* 63:510-527

Zhao Y, Tian X, Wang F, Zhang L, Xin M, Hu Z, Yao Y, Ni Z, Sun Q, Peng H (2017) Characterization of wheat MYB genes responsive to high temperatures. BMC Plant Biol 17:1-14

Zheng T, Hua C, Li L, Sun Z, Yuan M, Bai G, Humphreys G, Li T (2020) Integration of meta-QTL discovery with omics: Towards a molecular breeding platform for improving wheat resistance to Fusarium head blight. Crop J 9:739-749

Zhu T, Wang L, Rimbert H, Rodriguez JC, Deal KR, Oliveira RD, Choulet F, Keeble Gagnère GK, Tibbits J, Rogers J, Eversole K, Appels R, Gu YQ, Mascher M, Dvorak J, Luo MC (2021) Optical maps refine the bread wheat *Triticum aestivum* cv. Chinese Spring genome assembly. Plant J 107:303–314

Tables

Table 1 MQTLs detected for thermotolerance and associated traits in wheat

MQTL (CI range))	Flanking marker	LOD value	PVE (%)	^b Trait (No. of QTL)
MQTL1A.1 (33.4-59.2)	P3615.2/BobWhite_c863_1064	3.20	10.00	BMQ (2)
MQTL1A.2 (82.9-91.6)	RAC875_c25125_210/gwm905	5.40	14.87	SG (1), GW (1)
MQTL1A.3 (99.7-101.8)	gdm33/gwm33a	2.67	6.10	CF (2), GW (1)
MQTL1A.4 (105.6-107.7)	gwm33a/gwm1104	4.44	13.09	GN (1), GW (1), CF (1), GY (1), BMQ (3)
MQTL1A.5 (136.8-139.2)	Tdurum_contig11756_264/gwm691	5.04	12.00	GW (1), BMQ (1)
MQTL1B.1 (33.2-42.1)	RFL_Contig293_498/Excalibur_rep_c15165_367	3.69	22.60	HSI (1), BMQ (1), CF (1)
MQTL1B.2 (100.2-100.6)	wpt-0944/cwm90	3.50	15.42	GY (1), CC (2), GY (1), GFD (1), BMQ (1)
MQTL1B.3 (105.4-111.7)	wpt-0944/D_contig37179_75	3.54	9.40	FLD (1), GFD (1)
MQTL1B.4 (120.8-120.9)	wsnp_Ex_c1137_2183723/wsnp_Ku_c7822_13408189	3.91	12.17	BMQ (3), GW (1), TT (1), PT (1), SG (1), HSI (1)
MQTL1D.1 (117.4-134.6)	1228670s/BS00000721_51	4.15	13.50	BMQ (2)
MQTL1D.2 (213.9-216.9)	Excalibur_c16851_835/Kukri_c8533_1398	3.02	12.84	GFD (1), PMD (1), CC (1), TMD (1)
MQTL1D.3 (224.5-248.0)	Kukri_cA592_935/Ku_c17846_363	3.08	7.90	FLD (1), GY (1)

MQTL2A.1 (33.7-39.7)	wmc382b/barc124	5.00	17.13	GFD (2) (1), GN (1), BMQ (1)
MQTL2A.2 (56.0-65.3)	AX-111714220/Ra_c69660_1577	5.02	5.00	DTM (1), PH (1), NDVI (1)
MQTL2A.3 (87.8-89.8)	Ra_c31463_148/RAC875_c54668_102	8.35	16.97	BMQ (2), GW (1), WX (1), GN (1)
^a MQTL2A.4 (95.7-96.4)	Excalibur_c38761_288/BS00104558_51	5.62	10.32	BMQ (4), GW(2), PH (1)
MQTL2B.1 (73.8-75.2)	979087S/BS00075855_51	6.17	7.78	DTM (1), NDVI (1), BMQ (2), GN(2), CT (1)
MQTL2B.2 (119.6- 125.9)	BobWhite_c19433_185/1243120D	7.59	16.66	GY (1), SG (2), GW (3), GN (2), HSI (1), CF (1), FLD (1), CC (1), CT (1), BMQ (2), GFD (2), GY (1)
MQTL2B.3 (140.7- 145.9)	1125044D/wmc764	4.10	10.54	BMQ (6), NDVI (1), PMD (1), GY (1), GW (1), CF (1), TT (1)
MQTL2B.4 (158.1- 163.7)	wPt-2106/wPt-4195	3.39	9.46	CC (1), GY (1)
MQTL2B.5 (332.8-	RAC875-c37639_94/gwm935	3.25	18.99	GW (1), GFD (1)

352.6)

MQTL2D.1 (112.4-115.4)	GENE-0644_42/RAC875_C4314_993	5.28	12.55	GW (2), DTH (1), CT (3), GY (1), DTM (1), BMQ (1), PH (1)
MQTL2D.2 (124.3-128.7)	Kukri_c11566_386/gdm87a	6.88	15.73	BMQ (4), NDVI (1), GY (4), CT (1), KD (1), DTM (1), KH (1), GW (3), DTA (1), DTH (1), HI (1), FLD (2), HSI (1), NDVI (1)
MQTL2D.3 (153.0-154.8)	D_GA8KES401CIV9Z_240/BS00022276_51	4.89	11.38	BMQ (5), GY (3)
MQTL3A.1 (82.6-117.5)	2278441D/BobWhite_c17386_221	3.85	11.50	BMQ (2)
MQTL3A.2 (145.8-148.3)	Excalibur_c21521_1318/Kukri_c35153_956	3.36	5.22	GY (2)
MQTL3A.3 (166.1-167.3)	Kukri_c35153_956/BobWhite_c15763_205	11.97	12.11	PL (1), SG (1), GY (1), GN (1)
MQTL3A.4 (189.8-190.1)	BobWhite_c15763_205/barc54	4.77	19.70	TT (1), HSI (1), SG (1)
MQTL3B.1 (57.3-60.0)	wsnp_Ex_c1097_2105209(R)/Td durum_contig44013_164	4.27	14.32	CT (1), BMQ (6), FLD (1), GN (1), SG (1),
^a MQTL3B.2	wPt-7472/wPt-10552	4.84	11.48	BMQ (2),

(84.9-86.3)

GY(4)
(1), CC
(1), SG
(1), CT
(1), GW
(1), BM
(1), HSI
(1), SD
(1), CT
(1)

MQTL3B.3 (106.9- 110.5)	Kukri_c639_2168/BS00110416_51	5.83	15.48	CT (1), GY (4), TT (1), GW (1)
MQTL3D.1 (67.7-74.3)	gwm383/RAC875_c35399_497	5.03	9.20	GY(3) (1), BMQ(4) (1), HSI (1)
MQTL3D.2 (140.2- 145.0)	wmc529/D_GBF1XID02GHPCU_167	4.84	6.29	NDVI (1), BMQ (1)
MQTL3D.3 (176.4- 177.3)	Kukri_rep_c96809_352/gwm114a	5.89	7.33	GN (1), NDVI (1), GY (1)
MQTL4A.1 (631.6- 634.7)	BS00018120_51/tplb0043j11_1190	8.42	2.50	GW (1), SP (1)
MQTL4A.2 (648.4- 649.1)	tplb0043j11_1190/BS00069271_51	6.31	8.49	WSC (1), GN (1), GW
MQTL4A.3 (676.5- 681.9)	cfa2256/mgb7	4.21	11.13	BMQ (4), GW(3)
MQTL4B.1 (70.7-76.7)	wsnp_Ex_rep_c73742_71687509(R)/wsnp_Ex_c30695_39579408(R)	4.38	9.33	GW (1), BMQ (1)
MQTL4B.2 (100.1- 106.1)	Tdurum_contig60051_169/wsnp_Ex_c18433_27269748	7.60	19.70	BMQ (1), DTM (1), GFD (1), GW (1), BM (1), PH (1), HI (1)
MQTL4B.3 (127.5- 130.3)	Kukri_c27037_112/barc340a	4.19	8.15	KH (1), PH (1)
MQTL4B.4 (134.7- 136.3)	Kukri_c27037_112/barc340a	14.77	7.44	DTH (1), SP (1), PH

				(1), GW (2), TT (1), GN (1)
MQTL4B.5 (146.1-149.7)	Kukri_c27037_112/barc340a	4.06	5.53	GY (2), GW (1)
MQTL4B.6 (157.1-159.5)	wPt-11509/wPt-4537	5.16	8.84	DTM (2), GFD (1), GW (1), GN (1), BM (1), SG (1), PH (1)
MQTL4D.1 (0-7.3)	IACX11246/CAP8_c2722_258	5.10	10.00	BMQ (2)
MQTL4D.2 (30.1-39.0)	GENE-2463_463/Kukri_c29521_123	3.15	10.00	BMQ (2)
MQTL4D.3 (58.9-62.8)	Ex_c16174_685/D_contig26950_309	3.47	7.52	PH (1), GY (2)
MQTL4D.4 (72.6-75.0)	barc334/wmc89	29.76	4.50	SP (1), PH (1)
MQTL5A.1 (347.7-350.8)	gwm865/gwm330	4.49	9.17	GW (1), CT (2), GFD (1)
MQTL5A.2 (358.1-360.4)	utv711/wmc189_1	4.13	15.97	GW (3), GFD (2), WX (1), BMQ (1), KD (1), PC (1), GN (2), PH (1)
MQTL5A.3 (380.0-380.4)	1146968S/2255033D	4.55	9.58	GY (1), CT (3), GW (3), WSC (1), PH (2)
MQTL5B.1 (105.8-112.5)	1032121S/Ra_c28545_196	5.87	15.00	FLD (1), DTA (1), GW (1)
MQTL5B.2 (200.3-202.2)	1026809D/BooWhite_c1973_383	5.69	10.19	TT (1), DTM (1)
^a MQTL5B.3 (216.9-	1026809D/BooWhite_c1973_383	4.63	11.70	GY (1), GW (1), PC (1),

217.5)				BMQ (5)
MQTL5B.4 (234.7- 241.1)	BooWhite_c1973_383/BooWhite_c20282_164	4.37	13.15	HSI (1), BMQ (1), SD (1), KH (1), DTM (1), DTH (1), HI (1), GN (1)
MQTL5B.5 (289.8- 304.5)	wsnp_Ex_c12127_19394952(R)/Excalibur_c80450_75	6.00	13.50	BMQ (2)
MQTL5D.1 (27.9-28.9)	BS00034658_51/D_GCE8AKX02GJQ22_146	7.73	13.81	DTH (1), GW (1), BMQ (1)
MQTL5D.2 (76.0- 102.0)	Ra_c21355_446/RAC875_rep_c113555_122	6.20	10.50	BMQ (2)
MQTL5D.3 (110.3- 118.9)	RAC875_rep_c113555_122/wsnp_Ex_c30078_39930134	6.69	10.72	NDVI (1), CT (1)
MQTL5D.4 (135.9- 142.7)	wsnp_Ex_rep_c69631_68583363/D_GDEEGVY01D91EB_346	6.78	10.90	HSI (1), NDVI (1)
MQTL5D.5 (331.1- 337.2)	GENE-4974_839/BobWhite_c9761_225	4.76	7.23	GY (1), KH (1), CT (1)
MQTL6A.1 (444.5- 447.0)	wsnp_Ra_rep_c79566_75592262(R)/gdm36b	3.11	7.33	BMQ (1), GW (2)
MQTL6A.2 (470.9- 472.2)	RZ876/GTG.ACGC-108	5.09	22.45	CF (1), SG (2), CC (1), TMD (1), BMQ (1)
MQTL6A.3 (537.7- 539.6)	wsnp_Ku_c1318_2624758(R)/TGCGCG349	7.40	10.49	PH (1), BMQ (1)
MQTL6B.1 (145.4- 147.4)	barc211/BobWhite_c11652_421	2.85	9.71	CF (1), BMQ (1), SG (2)
MQTL6B.2 (159.0- 162.8)	BobWhite_c32377_278/wPt-689	13.41	20.28	BMQ (1), GN (1)

MQTL6B.3 (180.3-192.4)	RAC875_c16962_288/wmc419	3.26	9.33	GY (2), BMQ (1)
MQTL6B.4 (195.7-199.7)	wmc486/wsnp_Ex_c6057_10611952(R)	4.45	13.00	PT (1), GY (1)
MQTL6B.5 (692.8-704.8)	wsnp_JD_c1119_1642176/IACX3288	5.03	11.67	BMQ (1)
^a MQTL6D.1 (53.7-54.6)	CAP11_rep_c6864_291/Ra_c23837_298	12.28	26.45	GW (2), GFD (1), CT (3), GY (1)
^a MQTL7A.1 (94.5-96.2)	wPt-6460/gwm1187	4.24	14.74	SG (2), HSI (1), BMQ (3), GY (1)
MQTL7A.2 (97.8-98.3)	gwm1187/EXCALIBUR_C1625_1215	3.01	5.00	PC (1), GY (2)
MQTL7A.3 (104.1-108.4)	gwm1187/EXCALIBUR_C1625_1215	4.57	8.33	FLD (1), GY (2)
MQTL7A.4 (110.6-111.9)	gwm1187/EXCALIBUR_C1625_1215	3.95	4.89	TT (1), GN (1), GW (1)
MQTL7A.5 (115.3-116.3)	EXCALIBUR_C1625_1215/wPt-2	4.18	17.37	PH (1), GW (1), DTM (1)
MQTL7A.6 (122.1-124.5)	wPt-5153/wPt-5533	5.17	19.18	DTH (1), CF (2), PMD (1), CC (1), TMD (1), GN (1)
MQTL7A.7 (152.3-154.8)	RAC875_c9506_343/Kukri_c38390_218	5.25	12.75	HSI (1), CT (1), GFD (1), GW (1), DTH (1), BMQ (1)
^a MQTL7B.1 (79.9-80.6)	psr129/Ex_c24068_652	8.56	11.97	GY (3), HI (1), DTM (2), BM (1), GFD

					(1), GW (1), NDVI (1), DTH (1), GN (1)
MQTL7B.2 (101.4- 104.1)	wmc546.1/1102954 F 0-30:C>T-30:C>T	3.00	4.10	TT (1), CF (1)	
^a MQTL7B.3 (120.8- 122.4)	1220186D/GENE-4507_56	4.18	13.89	BMQ (6), GW (1), PH (1), GY (2) (1), CC (1), HSI (1), CT (1)	
MQTL7D.1 (44.5-71.2)	BobWhite_c28137_293/gwm332B	5.15	10.00	GY (1), BMQ (1)	
MQTL7D.2 (232.2- 245.7)	RAC875_rep_c105462_468/gwm535_1	3.36	9.85	HI (1), BMQ (1)	
MQTL7D.3 (286.4- 287.6)	gwm3100/Tdurum_contig74815_800	3.20	11.43	WSC (1), SG (1), BMQ (1), GW (1), DTM (1)	
MQTL7D.4 (312.8- 318.2)	wmc473_2/wmc94	4.79	8.13	SD (1), CF (1), DTH (1), GY (1), KH (1), GW (1), GN (1), DTM (1)	
MQTL7D.5 (354.1- 354.8)	RAC875_c39217_314/gwm1123	3.85	5.95	DTH (1), PH (1), WX (1)	
MQTL7D.6 (377.1- 383.4)	gwm350/Kukri_c37802_1215	7.04	11.55	DTM (1), NDVI (1)	

^aBreeder's QTL, ^bTrait abbreviations are available in Online Resource 6.

Table 2 Co-location of MQTLs with known genes for thermotolerance in wheat

Chr.	MQTL	Co-located known gene (physical coordinate, in bp)	Encoded protein	Reference
3D	MQTL3D.1	TaHSP17 (68325094-68325579)	Small heat shock proteins	Kumar et al. (2012)
3D	MQTL3D.1, 3D.3	TaHSP101 (379536976-379539614)	Heat shock protein	Campbell et al. (2001)
4A	MQTL4A.3	TaGF14-JM22 (580906528-580908057)	14-3-3 protein-like	Guo et al. (2018)
5B	MQTL5B.1	TaFER-5B (278795539-278797502)	Ferritin	Zang et al. (2017)
5B	MQTL5B.1	TaPEPKR2 (424332735-424335643)	Phosphoenolpyruvate carboxylase kinase-related kinase	Zang et al. (2018)
5D	MQTL5D.4	TaHsfA6f (461911512-461912559)	Heat shock protein	Xue et al. (2014)
7A	MQTL7A.2-7A.5	TaHSP90 (217556415-217557203)	Heat shock protein	Vishwakarma et al. (2018)

Table 3 High confidence candidate genes (CGs) underlying MQTLs for thermotolerance

MQTL designation	Gene ID (expression pattern)	GO term	Functional description
MQTL1A.1	TraesCS1A02G009320 (↓)	ADP binding	NB-ARC
MQTL1A.1	TraesCS1A02G010400 (↓)	ADP binding	Leucine-rich repeat
MQTL1A.3	TraesCS1A02G016200 (↓)	ADP binding	NB-ARC
MQTL2A.1	TraesCS2A02G005500 (↑)	integral component of membrane	Late embryogenesis abundant protein
MQTL2A.1	TraesCS2A02G007500 (↓)	integral component of membrane	Proton-dependent oligopeptide transporter family
MQTL2A.2	TraesCS2A02G085000 (↑) (↓)	integral component of membrane	WD40 repeat
MQTL2A.3	TraesCS2A02G093700 (↓)	protein serine/threonine phosphatase activity	PPM-type phosphatase domain
MQTL2B.1	TraesCS2B02G067000 (↓)	integral component of membrane	Protein kinase domain
MQTL2B.5	TraesCS2B02G539600 (↑) (↓)	membrane	Glycosyl transferase
MQTL3A.4	TraesCS3A02G454600 (↑) (↓)	DNA binding	Homeobox domain
MQTL3A.4	TraesCS3A02G455000 (↑)	-	Alpha/Beta hydrolase fold
MQTL3A.4	TraesCS3A02G456200 (↑)	-	Alpha/beta hydrolase fold-1
MQTL3A.4	TraesCS3A02G456300 (↓)	-	Alpha/beta hydrolase fold-1
MQTL3D.1	TraesCS3D02G127000 (↓)	protein binding	F-box domain
MQTL3D.2	TraesCS3D02G037200 (↓)	lipid binding	C2 domain
MQTL3D.2	TraesCS3D02G037600 (↓)	structural constituent of ribosome	Ribosomal protein S10
MQTL3D.2	TraesCS3D02G037900 (↓)	regulation of systemic acquired resistance	NPR1/NH1-interacting protein
MQTL4A.1	TraesCS4A02G016900 (↑) (↓)	oxidoreductase activity	3-dehydroquinate synthase
MQTL4A.1	TraesCS4A02G017700 (↓)	nucleus	Zinc finger, Dof-type
MQTL4A.1	TraesCS4A02G019000 (↓)	structural constituent of ribosome	Ribosomal protein
MQTL4B.6	TraesCS4B02G041800	integral component of membrane	SNARE associated Golgi

	(↓)		protein
MQTL4D.3	TraesCS4D02G008500 (↓)	hydrolase activity, acting on ester bonds	GDSL lipase/esterase
MQTL5B.1	TraesCS5B02G412200 (↑) (↓)	ATP binding	Protein kinase domain
MQTL5B.1	TraesCS5B02G412900 (↓)	hydrolase activity	Pectinacetyl esterase/NOTUM
MQTL5D.1	TraesCS5D02G205200 (↓)	nucleic acid binding	RNA recognition motif domain
MQTL5D.2	TraesCS5D02G217000 (↓)	rRNA binding	Brix domain
MQTL5D.5	TraesCS5D02G339700 (↑) (↓)	integral component of membrane	Protein kinase domain
MQTL5D.5	TraesCS5D02G340000 (↑) (↓)	regulation of protein catabolic process	Proteasome component (PCI) domain
MQTL6A.1	TraesCS6A02G022100 (↓)	transferase activity, transferring acyl groups other than amino-acyl groups	Transferase
MQTL6D.1	TraesCS6D02G328700 (↑) (↓)	-	Zinc finger, RING-type
MQTL7A.1	TraesCS7A02G287000 (↑) (↓)	RNA binding	K Homology domain, type 2
MQTL7A.5	TraesCS7A02G193000 (↓)	protein binding	Ankyrin repeat
MQTL7A.7	TraesCS7A02G184700 (↑) (↓)	ATP binding	AAA+ ATPase domain
MQTL7D.2	TraesCS7D02G049300 (↓)	integral component of membrane	Proton-dependent oligopeptide transporter family
MQTL7D.4	TraesCS7D02G011800 (↓)	ATP binding	Protein kinase domain
MQTL7D.4	TraesCS7D02G014900 (↓)	nucleus	SANT/Myb domain

GO gene ontology, ↑ up-regulated genes, ↓ down-regulated genes, ↑↓ up-regulated under at one time-point and down-regulated at other

Table 4 Identification of ortho-MQTLs between wheat and rice genomes

Ortho-MQTL designation	Wheat MQTL (physical position, in Mb) ^a	Rice MQTL (chromosome; physical position, in Mb) ^b
Ortho-MQTL1B.4	MQTL1B.4 (7.33-9.33)	rMQTL5.3 (5; 24.26-27.34)
Ortho-MQTL2B.3	MQTL2B.3 (580.34-582.34)	rMQTL4.4 (4; 26.86-29.06)
Ortho-MQTL2D.1	MQTL2D.1 (53.8-55.8)	rMQTL7.1 (7; 28.41-29.47)
Ortho-MQTL2D.3	MQTL2D.3 (26.18-28.18)	rMQTL4.3 (4; 21.45-24.16); rMQTL4.4 (4; 26.86-29.06)
Ortho-MQTL3B.2	MQTL3B.2 (9.74-11.74)	rMQTL1.2 (1; 34.95-36.73)
Ortho-MQTL7B.3	MQTL7B.3 (82.78-84.78)	rMQTL6.3 (6;16.75-19.34); rMQTL6.2 (6;3.17-5.09); rMQTL8.2 (8;22.47-25.59)
Ortho-MQTL7D.4	MQTL7D.4 (4.55-6.55)	rMQTL8.1 (8; 3.93-9.78)

^a Genomic position of MQTL peak region (total 2 Mb region)

^b Raza et al (2020)

Figures

Figure 1

Characteristic features of the collected QTLs and their distribution at different levels. (a) Number of QTLs in each study. (b) Number of QTL in each sub-genome of wheat. (c) PVE and LOD score of QTLs. (d) Trait-wise distribution

of number of QTLs. Traits abbreviations include GY grain yield, GW grain weight, GN grain number, DTH days to heading, DTA days to anthesis, DTM days to maturity, GFD grain filling duration, HI harvest index, BM biomass, PT productive tiller, KD kernel diameter, SL spike length, SD spike density, SPP spikes per plant, PH plant height, BMQ bread making quality traits, SG stay green, CF chlorophyll fluorescence, CC chlorophyll content, CT canopy temperature, NDVI normalised difference vegetation index, HSI heat susceptibility index, TT thermotolerance, FLD flag leaf dimension, WX waxiness, TMD thylakoid membrane damage, KD kernel hardness, PL length of the ear peduncle, PMD plasma membrane damage, WSC water soluble carbohydrates, and PC proline content

Figure 2

Distribution of Meta-QTLs (MQTLs) on all the 21 wheat chromosomes. Only the flanking markers are shown for better visualization. MQTLs in blue boxes (right side) were co-located with MTAs identified using genome-wide

association studies (GWAS)

Figure 3

Heat maps representing differential expression of some important genes underlying 33 MQTLs (shown on the left). The scale drawn on the top of heat maps exhibits the level of expression. Proteins which are known to be associated with thermotolerance are marked by an asterisk (*)

Figure 4

Representing syntenic regions of two ortho-MQTLs between wheat and rice. The chromosome number, physical position, and common genes between the wheat and rice are indicated. More details are presented in Online Resource 12

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryInformation1.xlsx](#)
- [SupplementaryInformation2.docx](#)