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Meta-QTLs, Ortho-metaQTLs and Candidate Genes for Grain Yield and Associated Traits in Wheat (*Triticum Aestivum* L.)

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Abstract

Meta-QTL analysis was conducted using 8,998 known QTLs, which included 2,852 major QTLs for grain yield (GY) and its following ten component/related traits: (i) grain weight (GWei), (ii) grain morphology related traits (GMRTs), (iii) grain number (GN), (iv) spikes related traits (SRTs), (v) plant height (PH), (vi) tiller number (TN), (vii) harvest index (HI), (viii) biomass yield (BY), (ix) days to heading/flowering and maturity (DTH/F/M) and (x) grain filling duration (GFD). The QTLs used for this study were retrieved from 230 reports (including 19 studies conducted in tetraploid wheat) that were based on 190 mapping populations (1999–2020). The study resulted in the identification of 141 meta-QTLs (MQTLs), with an average confidence interval (CI) of 1.37 cM (reduced 8.87 fold), the average CI in the initial QTLs being > 12.15 cM. As many as 63 MQTLs, each based on at least 10 initial QTLs were stable and robust; with 13 MQTLs are described as breeder's QTLs. MQTLs were also utilized for the identification of 1,202 candidate genes (CGs), which included 18 known genes. The MQTLs were also found to contain 50 wheat genes that were homologous to 35 known yield-related genes from rice, barley, and maize. Further, the use of synteny and collinearity allowed the identification of 24 ortho-MQTLs which were common among the wheat, barley, rice, and maize. The results of the present study should prove useful for wheat breeding and future basic research in cereals including wheat, barley, rice, and maize. In particular, the breeder's QTLs can be used for marker-assisted selection for grain yield and fine mapping leading to cloning of QTLs/genes for yield and related traits.

Key Message

The study used 8,998 QTLs which involved 2,852 major QTLs reported in earlier studies in wheat and identified 141 meta-QTLs (MQTLs), which include 13 MQTLs described as breeder's QTLs, 24 ortho-MQTLs, 1,202 candidate genes, and 50 wheat homologues of genes for grain yield from rice, maize, and barley.

Introduction

Wheat provides approximately 20% of calories in the human diet worldwide; therefore, it is the most important crop for food and nutritional security (Shiferaw et al. 2013). The global annual wheat production during the last five decades has been rising steadily, and therefore, we have witnessed a global wheat production of 750 million metric tons in the year 2020 (https://knoema.com/atlas/topics/Agriculture/Crops-Production-Quantity-tonnes/Wheat-production). According to most estimates, the production should increase by ~ 50% during the next three decades (https://www.openaccessgovernment.org/demand-for-wheat/83189/) to meet the demand of the growing population, which is expected to reach 9 to 10 billion by 2050 (Shiferaw et al. 2013; Yadav et al. 2019). Further improvement in yield potential is needed to improve and sustain the required annual growth rate of around 2%. This will be possible only through a further detailed understanding of the genetic architecture of grain yield and associated traits (Gupta et al. 2020).

Grain yield is widely known to be a complex quantitative trait, which is controlled by a large number of QTLs/genes. Major yield contributing traits include the following: grain number, grain weight, grain morphology-related traits, tiller number, spike-related traits, harvest index, plant height, and heading date (Gupta et al. 2020; Hu et al. 2020). Therefore, these traits are continuously targeted in wheat breeding programmes for the development of novel high-yielding varieties (Zhou et al. 2007). More recently, the markers associated with QTLs/genes for these traits have also been exploited for marker-assisted selection (MAS), although this use has been minimal due to the non-availability of major and robust QTLs (Misztal, 2006; Collard and Mackill, 2008). The meta-QTL analysis is an approach that has been shown to provide more robust and reliable QTLs, including 'QTL hotspots' (Goffinet and Gerber, 2000; Salvi and Tuberosa, 2015). The precision of meta-QTL analysis has been further improved due to the development of new algorithms (Arcade et al. 2004; Veyrieras et al. 2007; de Oliveira et al. 2014).

MQTLs in wheat have already been identified for several traits, including the following: (i) ear emergence (Hanocq et al. 2007), (ii) pre-harvest sprouting tolerance (Tyagi and Gupta, 2012), (iii) resistance to Fusarium head blight (Venske et al. 2019), (iv) tolerance to heat stress (Acuña-Galindo et al. 2015; Kumar et al. 2020) and (v) yield and quality-related traits (Griffiths et al. 2009; Gegas et al. 2010; Zhang et al. 2010; Griffiths et al. 2012; Quraishi et al. 2017; Bilgrami et al. 2020; Liu et al. 2020). Regular discovery of more QTLs and improvements in algorithms for more precise meta-QTL analysis calls for an update on the same.

Keeping the above in view, meta-QTL analysis was conducted on grain yield (GY) and a number of its contributing traits listed above. For this purpose, we utilized data from 230 studies published from 1999 to 2020 (Table S1). The MQTLs identified during the present study were also used to identify CGs. In addition, due to high synteny and collinearity among cereals including wheat, rice, maize, and barley (Kumar et al. 2009; Mayer et al. 2011; Hirsch et al. 2014), we expanded our analysis to detect ortho-MQTLs among these cereals. Genomic regions associated with MQTLs that are homologous to known genes for yield in other cereals (rice, barley, and maize) were also identified. We believe that this work should prove useful not only for molecular breeding but also for basic research on structural genes and regulatory elements (including fine mapping and cloning of QTLs) involved in grain yield and associated traits not only in wheat but also in other cereals.

Materials And Methods

Collection of QTLs data for yield and associated traits in wheat

The literature related to QTL mapping of grain yield and its component traits was collected from PubMed (http://www.ncbi.nlm.nih.gov/pubmed) and Google Scholar (https://scholar.google.com/) using appropriate keywords. For each QTL, the following data were collected: (1) QTL name, wherever available, (2) flanking markers or closely linked marker, (3) peak position and confidence interval, (4) type and size of the mapping population used, (5) LOD score, and (6) phenotypic variation explained (PVE) or R^2 value**. In some cases, where peak position was missing, the mid-point between the two flanking markers was**

treated as the peak. Secondly, when an actual LOD score for an individual QTL was not available, but test statistic was given, LOD score was calculated using the available test statistic; if no information was available, a LOD score of 3.0 was treated as the threshold for the current study.

If names of QTLs were not available, names were assigned following the standard nomenclature (letter "Q" followed by the abbreviated name of the trait, the institute involved, and the chromosome). Different QTLs on the same chromosome were distinguished by using Arabic numerical identifiers following each specific chromosome. All QTLs were accommodated in the following traits: (i) 'grain weight' (GWei): recorded as thousand-grain weight, 50-grains weight, mean grain weight, hundred-grain weight, single grain weight, grain weight per plant, and test weight, (ii) 'grain number' (GN): recorded as average grain number per spike, grain number per spike, grain number per square meter, grain number per spikelet, grains per spikelet, and grains per fertile spikelet, etc; (iii) 'grain morphology related traits' (GMRTs): recorded as grain length, grain width, grain length-width ratio, grain thickness, grain thickness-length ratio, grain area, and grain diameter, grain volume weight, etc.; (iv) 'spike related traits' (SRTs): recorded as spike length, spikes per plant, spikes per square meter, spike compactness, spike formation rate, spike layer uniformity, basal sterile spikelet number, top sterile spikelet number, fertile floret per spike, and spikelets per spike, etc. (v) 'biomass yield' (BY): recorded as total biomass, tiller biomass, and plant biomass; (vi) 'tiller number' (TN): recorded as effective tiller number, tiller number per plant, and tiller number per square meter. Whereas, some traits were treated individually by the available name of the trait (e.g., heading, days to flowering, days to maturity, grain filling duration, earliness per se, and plant height).

Construction of consensus linkage map

A consensus map was developed using the following seven available linkage maps involving different types of markers, which have been widely used in QTL mapping studies: (i) 'Wheat_Composite_2004' with 4403 marker loci, available at GrainGenes database (http://wheat.pw.usda.gov**); (ii) the 'Wheat, Consensus SSR, 2004' with 1235 marker loci (Somer et al. 2004); (iii) an integrated map for durum wheat with 3669 markers (Marone et al. 2013); (iv) four SNP maps developed using following SNP arrays: 'Illumina 9K iSelect Beadchip Array' (Cavanagh et al. 2013), 'Illumina iSelect 90K SNP Array' (Wang et al.2014), 'Wheat 55K SNP array' (Winfield et al. 2016) and the 'AxiomR, Wheat 660K SNP array' (Cui et al. 2017). Marker information or maps from several other independent studies were also included for developing the consensus map.**

The R package LPMerge was employed for the construction of the consensus map (Endelman and Plomion, 2014), which involves the following two steps: (i) first, it calculates the number of consensus bins, number of markers, and the initial number of ordinal conflicts. If the linkage maps have inconsistencies in the order of markers, it resolves them by removing ordinal constraints using certain statistical manipulations; (ii) then, it develops 1 to 4 consensus maps as desired (K = 1 to 4, where K is the maximum interval size); of which one can select one or more maps using the associated statistics developed for this purpose; this is done through estimation of root mean square error (RMSE, based on mean and standard deviation, sd) between each individual map and the consensus map. The consensus

map with a length close to the mean length of the component linkage maps associated with the lowest RMSE was accepted as the final map for further analysis.

QTL projection and meta-QTL analysis

From all the collected QTLs, only major QTLs, each explaining ≥ 10% of phenotypic variation for the target trait were selected for further analysis. Confidence interval (CI, 95%) was estimated for each of these QTLs, through following different population-specific equations: (i) for recombinant inbred lines (RILs): CI = 163/ (population size x R^2); (ii) for F_2 and backcross populations: CI = 530/(population size x R^2); and (iii) for doubled haploid (DH): CI = 287/(population size x R^2); in these three equations, 163, 530 and 287 are the population-specific constants obtained from different simulations (**Darvasi and Soller, 1997; Guo et al., 2006; Venske et al. 2019**). The selected QTLs each with the required information (estimated CIs, peak positions, initial LOD score, and R^2 or PVE value) were projected onto the consensus map using BioMercator V4.2 (**Sosnowski et al. 2012**) (Table S1). Following this projection, meta-analysis was performed, for each chromosome individually, via the Veyrieras two-step algorithm available in the software. The best meta-QTL model was selected when the lowest values of the model selection criteria were achieved in at least three models; the selection criteria used for this purpose included the following: Akaike information content (AIC), Akaike correction (AICc), AIC model 3 (AIC3), Bayesian information criteria (BIC), and Average weight of evidence (AWE). The statistical procedures and algorithms available in this software are available in Sosnowski et al. (2012).

Candidate genes (CGs) within MQTLs and their expression analysis

For identifichttps://wheat.pw.usda.gov/GG3https://www.cerealsdb.uk.net/cerealgenomics/CerealsDB/indexNEW.phphttps://wheat-urgi.versailles.inra.fr/

Further, we conducted an *in-silico* transcriptional analysis for the identified putative CGs using the 'Wheat Expression Browser-expVIP' (expression Visualization and Integration Platform) (http://www.wheat-expression.com) (Ramírez-González et al. 2018). Gene expression datasets relevant to the present study were utilized for this purpose; these datasets includes the following: 'Developmental time-course of Chinese Spring (Choulet et al. 2014), 'synthetic hexaploid' (Li et al. 2014); 'Grain tissue-specific developmental time-course' (**Gillies et al. 2012; Pearce et al. 2015; Pfeifer et al. 2014); 'Chinese spring leaves and roots at both seedling (Clavijo et al. 2017), seven leaf stages' (Ramírez-González et al. 2018) and 'Gene expression during a time-course of flag leaf senescence' (Borrill et al. 2019).** Following the criteria proposed by Wagner et al. (2013), only CGs showing at least 2 transcripts per million (TPM) expression were considered for the present study. Heat maps for expression data were constructed using the software 'Morpheus' (https://software.broadinstitute.org/morpheus/).

A search was also made to collect information regarding various wheat genes related to the traits in question. Nucleotide sequences of these genes were retrieved from the NCBI database (https://www.ncbi.nlm.nih.gov/) using accession IDs given in the corresponding studies. BLASTN searches were then carried out against the genomic database (available in EnsemblPlants) of wheat to

find the physical positions of these genes in the genome. These physical coordinates of the genes were compared with the physical intervals of the MQTL regions; an individual known gene falling within a specific MQTL region was considered as the MQTL region co-located with the corresponding gene.

MQTL regions homologous to known genes from other cereals

Information on rice, barley, and maize genes associated with grain yield and related traits was collected from the literature. Amino acid sequences for these genes were retrieved from the NCBI (https://www.ncbi.nlm.nih.gov/) and used for BLASTP searches to identify the corresponding wheat proteins (available in EnsemblPlants) at an E-value of $<10^{-10}$, with 60% coverage, and >60% identity. Physical positions of the corresponding genes and wheat MQTLs were then compared to detect the MQTL regions homologous to known genes from other cereals.

Results

QTLs associated with different traits

A total of 8,998 QTLs were available from 230 studies, which involved 190 mapping populations. The size of the mapping populations ranged from 32 to 547 DH/RIL lines; these mapping populations also included 26 F2/BC populations. As many as 19 studies involved durum wheat, mainly published during 2015–2020 (for details, see Table S1). The details of the data on QTLs for different traits and their distribution on genomes and chromosomes are presented in Table S2 and Fig. 1.

The selected 2,852 QTLs were distributed on all the 21 wheat chromosomes with a range of 39 QTLs on 1D to 210 on 2B (Fig. 1b). Of the QTLs used for analysis, the number of QTLs in three sub-genomes differed [1,084 (38%) QTLs on sub-genome A, 1,114 (39.06%) on sub-genome B, and 653 (19.74%) on sub-genome D (Table S2)]. The number of QTLs per trait also differed with a range of 50 for TN to 768 for SRTs (Table S2). LOD scores for these QTLs ranged from 1.7 to 130.48 (Chen et al. 2020) with 45.75% of QTLs showing a LOD score from 3 to 5 (Fig. 1c). The percentage of phenotypic variation explained (PVE) by individual QTL ranged from 10 to 98.7% (average of 17.82%), with most (49.92%) of the QTLs showing a PVE less than 15 % (Fig. 1d). Most of the QTL data collected in this study has been included in the recently developed WheatQTL database (http://wheatqtldb.net/) (Singh et al. 2021).

Construction Of High-density Consensus Map

The integrated consensus map contained 2,33,856 markers, which included a variety of markers including the following types: SNPs, DArT, SSR, AFLP, RAPD, STS, EST-SSR, SRAP, ISSR, and KASP markers. Following important genes are also included on this consensus map: *Vrn, Ppd, Rht*, and *Glu* loci (Table S3). The total length of the consensus map is 11,638.76 cM; the length of the individual chromosomes ranged from 281.26 cM (4D) to 763.08 cM (4A) (Fig. 2). The average number of markers carried by an individual chromosome was 11,136 (Table S3, S4). The marker densities for individual

linkage groups ranged from 12.76 to 48.27 markers per cM for sub-genome A, from 16.71 to 33.48 for sub-genome B, and from 7.74 to 18.13 for sub-genome D (Fig. 2). Generally, the density of markers at one end of the chromosome was significantly higher than at the other end (Table S3, S4).

Qtl Projection And Meta-qtl Analysis

Only 1842 QTLs of the 2,852 QTLs that were initially selected for projection, could be projected onto the consensus map; the remaining 1,010 QTLs could not be projected, as either the associated markers were absent in the consensus map, or they had low R^2 values and/or large CI. The projection resulted in the identification of 141 MQTLs, derived from 1828 QTLs; the remaining 14 QTLs were single QTLs, which did not find a place in any MQTL (Table 1). The number of MQTLs differed in three different sub-genomes (38 in sub-genome A, 54 in sub-genome B, and 49 in sub-genome D), and also in 21 individual chromosomes (from 3 on chromosome 6A to 10 each on chromosomes 4D and 6B). These were named using sequential Arabic numerals for each chromosome (e.g. MQTL1A.1 to MQTL1A.7). Whereas, LOD score and PVE value of each MQTL were calculated as the mean value of the initial QTLs involved. The number of QTLs per MQTL ranged from 2 on each of several chromosomes to 71 on chromosome 5A (MQTL5A.2). Sixty-three MQTLs (each based on at least 10 initial QTLs) were considered to be the most stable and robust (independent from phenotyping environments and genetic backgrounds). The density of MQTLs did not match the density of initial QTLs on individual chromosomes. For instance, the number of MQTLs on chromosomes 4A, 5A, and 7A were low relative to the corresponding densities of initial QTLs. The CI for MQTLs ranged from 0.01 to 13.44 cM with an average CI of 1.37 cM, with 85 MQTLs each having a CI of < 1 cM (Table 1, S5).

Table 1
Yield-related MQTLs identified in the present study

Sr. no.	MQTL name (Total physical interval in Mb) [MQTL peak region]	Flanking markers (CI, in cM)	Initial QTLs (avg. LOD score)	Traits (avg. PVE)
1.	MQTL1A.1 (5.03–7.52)[b]	AX-110506772/AX-109884241 (21.58–22.51)	4 (8.41)	GWei, GY, and SRTs (12.37)
2.	MQTL1A.2 (16.44–32.89) [23.66–25.66]	IWB45602/AX-111475421 (30.39–31.33)	10 (6.76)	SRTs, GMRTs, and GWei (14.54)
3.	MQTL1A.3 (20.89–22.89)	AX-109896396/AX-95156759 (39.14–40.2)	11 (7.03)	GMRTs, GN, SRTs, GWei, and GY (22.75)
4.	MQTL1A.4 (23.13–24.32)	AX-109272204/AX-94547512 (45.62–46.48)	2 (9.15)	SRTs (21.30)
5.	MQTL1A.5 (23.81–26.53) [24.17–26.17]	AX-110597220/AX-111596418 (48.7–49)	23 (6.53)	SRTs, PH, GWei, GN, TN, GMRTs, DTF, and HI (17.92)
6.	MQTL1A.6 (41.74–42.26)	IWB64888/Xcfe26.5 (49.31–50.2)	2 (9.17)	GN (22.73)
7.	MQTL1A.7 (28.62–28.76)	AX-95129506/AX-94383313 (51.91–52.5)	2 (4.54)	SRTs and GMRTs (11.85)
8.	MQTL1A.8 (12.16–54.02) [32.08–34.08]	IWA4240/AX-94492795 (58.82–58.95)	23 (5.47)	GMRTs, GN, BY, SRTs, GY, GWei, and TN (16.27)
9.	MQTL1B.1 (2.33–6.35) [3.34–5.34]	IWB54702.1/IWB28576 (11.97–12.18)	8 (8.25)	SRTs and DTF (18.74)

GY grain yield, Gwei grain weight, GN grain number, GMRTs grain morphology related traits, SRTs spike related traits, BY biomass yield, HI harvest index, DTH days to heading, DTF days to flowering, DTM days to maturity, GFD grain filling duartion, EPS earliness per se, PH plant height, TN tiller number, Avg. Average, [a]'Breeder's QTLs (had high PVE values, LOD scores, and reduced CI), [b]MQTL peak region was not calculated for the MQTLs which had ≤ 2 Mb physical interval.

Sr. no.	MQTL name (Total physical interval in Mb) [MQTL peak region]	Flanking markers (CI, in cM)	Initial QTLs (avg. LOD score)	Traits (avg. PVE)
10.	MQTL1B.2 (5.99–99.6) [51.79–53.79]	AX-94888336/AX-95186655 (48.18–50.07)	4 (8.70)	GMRTs and GWei (16.69)
11.	MQTL1B.3 (9.55–9.71)	IWB57219/AX-95223114 (77.2877.9)	7 (7.52)	SRTs, and GMRTs (16.20)
12.	[a]MQTL1B.4 (10.07–16.89) [12.48–14.48]	IWB43001/IWB12258 (88.96–89.64)	6 (38.62)	TN, SRTs, GWei, and GMRTs (26.60)
13.	MQTL1B.5 (17.34–17.38)	IWB61919/IWB11925.1 (93.01–93.2)	45 (5.10)	GMRTs, SRTs, DTM, DTF, HI, GWei, GY, GN, TN, BY, GFD, and PH (14.52)
14.	MQTL1B.6 (12.81–13.25)	IWB47571/IWB8902 (95.73–96.06)	25 (7.12)	GN, DTH, SRTs, BY, GMRTs, DTF, and GWei (15.57)
15.	MQTL1B.7 (390.71–412.9) [400.80-402.80]	IWB8913/Ku_c20478_390 (103.4-104.59)	19 (5.86)	GWei, DTF, HI, SRTs, GMRTs, EPS, GY, GN (17.29)
16.	MQTL1B.8 (454.93-466.09) [459.50-461.50]	IWB35083/Xutv1391b (114.34-114.52)	14 (4.51)	GWei, SRTs, GMRTs, EPS, DTM, TN, GY and PH (12.72)
17.	MQTL1D.1 (0.91–2.08)	AX-95104817/XGli1 (15.75–25.05)	3 (6.78)	SRTs, TN, and DTF (16.18)

GY grain yield, Gwei grain weight, GN grain number, GMRTs grain morphology related traits, SRTs spike related traits, BY biomass yield, HI harvest index, DTH days to heading, DTF days to flowering, DTM days to maturity, GFD grain filling duartion, EPS earliness per se, PH plant height, TN tiller number, Avg. Average, [a]'Breeder's QTLs (had high PVE values, LOD scores, and reduced CI), [b]MQTL peak region was not calculated for the MQTLs which had ≤ 2 Mb physical interval.

Sr. no.	MQTL name (Total physical interval in Mb) [MQTL peak region]	Flanking markers (CI, in cM)	Initial QTLs (avg. LOD score)	Traits (avg. PVE)
18.	MQTL1D.2 (10.27–10.66)	IWA6621/IWB35174 (70.42–70.93)	6 (7.34)	GMRTs, SRTs, and GWei (12.74)
19.	MQTL1D.3 (6.54–10.81) [7.67–9.67]	IWB60500.2/AX-94653386 (76.32–83.79)	3 (6.61)	GY, GWei, and PH (14.82)
20.	MQTL1D.4 (28.5-36.31) [31.40–33.40]	P32/M59-2/1DS_1914495_3816 (95.78–96.28)	7 (5.06)	DTH, DTM, GWei and GN (15.00)
21.	MQTL1D.5 (7.87-18) [11.93–13.93]	XksuE18a/AX-94555171 (106.49-113.89)	3 (5.34)	SRTs, GWei, and PH (14.06)
22.	MQTL1D.6 (12.32–22.89) [16.60–18.60]	Xwmc63.1/IWB23920 (118.51-122.11)	3 (3.52)	GMRT and GWei (14.18)
23.	MQTL2A.1 (18.59–79.75) [48.168–50.16]	BS00021739_51/GENE-1031_48 (122.34-123.24)	19 (9.95)	SRTs, GMRTs GN, PH, GY, DTM, DTH, and GWei (17.77)
24.	MQTL2A.2 (206.86-508.73) [356.79-358.79]	IAAV1587/IWB14868 (142.74-143.76)	26 (10.67)	GMRTs GN, GWei, SRTs, HI, BY, PH, and DTH (15.19)
25.	MQTL2A.3 (21.31–63.36) [41.33–43.33]	AX-95140204/AX-109425280 (151.07-153.05)	12 (5.58)	SRTs, GWei, GN, and GMRTs (14.70)
26.	MQTL2A.4 (24.31–31.98) [27.14–29.14]	Xwmc630b/AX-108741878 (158.76-159.04)	4 (9.26)	SRTs, GN, and GWei (13.04)

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Sr. no.	MQTL name (Total physical interval in Mb) [MQTL peak region]	Flanking markers (CI, in cM)	Initial QTLs (avg. LOD score)	Traits (avg. PVE)
27.	[a]MQTL2A.5 (27.14–30.46) [27.79–29.79]	Ku_c3202_1646/IWB43373 (162.13-162.23)	4 (39.33)	GWei, and GMRTs (34.67)
28.	MQTL2A.6 (30.46–31.99)	IWB43373/tPt-9405 (162.64-163.12)	20 (6.38)	GY, GN, DTH, DTM, SRTs, GMRTs and GWei (20.41)
29.	MQTL2A.7 (36.94–42.19) [38.56–40.56]	Ppd-A1/Xgpw2204 (187.13-187.29)	7 (4.18)	SRTs, GWei, TN, and GMRTs (13.38)
30.	MQTL2B.1 (3.41–24.26) [12.83–14.83]	IWB40903/IWB17530 (72.48–73.36)	10 (7.29)	SRTs, GN, GMRTs GY, and GWei (12.66)
31.	MQTL2B.2 (25.28–26.3)	IWB65267.1/IWB41081 (83.24–84.5)	6 (6.63)	SRTs, GMRTs and GWei (14.44)
32.	MQTL2B.3 (28.37–29.99)	IWB65370.2/IWB45990 (102.57-103.78)	31 (7.07)	SRTs, DTM, GN, GMRTs, GY, HI, GWei, DTH, DTF, and PH (15.92)
33.	MQTL2B.4 (29.16–30.5)	IWB35771/IWB30649 (114.43-114.98)	16 (10.55)	HI, DTH, GN, GMRTs, SRTs, PH, and DTF (16.21)

GY grain yield, Gwei grain weight, GN grain number, GMRTs grain morphology related traits, SRTs spike related traits, BY biomass yield, HI harvest index, DTH days to heading, DTF days to flowering, DTM days to maturity, GFD grain filling duartion, EPS earliness per se, PH plant height, TN tiller number, Avg. Average, [a]'Breeder's QTLs (had high PVE values, LOD scores, and reduced CI), [b]MQTL peak region was not calculated for the MQTLs which had ≤ 2 Mb physical interval.

Sr. no.	MQTL name (Total physical interval in Mb) [MQTL peak region]	Flanking markers (CI, in cM)	Initial QTLs (avg. LOD score)	Traits (avg. PVE)
34.	MQTL2B.5 (7.11–30.5) [17.80–19.80]	IWB64873/IWB28040 (118.09-118.26)	31 (7.05)	GMRTs, GWei, GN, GY, BY, SRTs, PH, DTH, and HI (17.29)
35.	MQTL2B.6 (7.77–11.08) [8.42–10.42]	IWB26439/wPt-0746 (126.62-127.31)	35 (9.50)	SRTs, GMRTs, DTF, GY, DTH, DTM, GN, GWei, HI, TN, and PH (17.64)
36.	MQTL2B.7 (42.28–59.18) [49.72–51.72]	AX-94911223/Xwmc617.1 (170.03-170.04)	7 (7.02)	GN, PH, GY, SRTs, and GWei (15.37)
37.	MQTL2D.1 (1.67–5.32) [2.85–4.85]	Xbarc114/AX-94611751 (0.1–0.65)	9 (8.70)	GY, SRTs, HI, DTH, DTM, and DTF (19.75)
38.	MQTL2D.2 (9.58–10.32) []	3033925|F|0/3029203|F|0 (6.8–7.36)	16 (8.40)	GWei, GMRTs, GY, PH, SRTs, and DTH (17.59)
39.	MQTL2D.3 (16.36–28.09) [21.22–23.22]	PPD-D1/AX-110595669 (35.55–48.99)	3 (41.13)	DTH and SRTs (32.13)
40.	MQTL2D.4 (14.9-28.87) [20.88–22.88]	AX-110668803/2DS_5365757_8955 (59.46–61.31)	9 (6.22)	SRTs, GWei, GY, GMRTs, PH, HI, and GN (14.92)

GY grain yield, Gwei grain weight, GN grain number, GMRTs grain morphology related traits, SRTs spike related traits, BY biomass yield, HI harvest index, DTH days to heading, DTF days to flowering, DTM days to maturity, GFD grain filling duartion, EPS earliness per se, PH plant height, TN tiller number, Avg. Average, [a]'Breeder's QTLs (had high PVE values, LOD scores, and reduced CI), [b]MQTL peak region was not calculated for the MQTLs which had ≤ 2 Mb physical interval.

Sr. no.	MQTL name (Total physical interval in Mb) [MQTL peak region]	Flanking markers (CI, in cM)	Initial QTLs (avg. LOD score)	Traits (avg. PVE)
41.	MQTL2D.5 (14.36–88.35) [50.35–52.35]	AX-94750495/wPt-0330 (67.72–68.15)	35 (7.62)	GWei, GN, GY, GMRTs, SRTs, HI, PH, DTH, and DTM (15.93)
42.	MQTL2D.6 (14.9-21.06) [16.98–18.98]	2DS_5390826_7647/Estssr-143 (76.43–76.84)	38 (8.96)	GMRTs, DTM, DTH, GWei, SRTs, PH, BY, GY, and GN (18.07)
43.	MQTL2D.7 (23.42–25.04)	Xfba400/AX-94743556 (89.87–90.61)	36 (9.07)	SRTs, GWei, GMRTs PH, DTH, GY, and DTM (19.11)
44.	MQTL2D.8 (20.77–578.7) [298.73-300.73]	AX-110939188/JD_c63957_1176 (100.1-100.15)	11 (11.31)	GMRTs, TN, GN, GWei, GY, PH, and SRTs (14.73)
45.	MQTL3A.1 (7.48–9.11)	IWB73673/Xgpw7080 (40.14–40.52)	11 (6.09)	SRTs, PH, GMRTs, GWei, DTM, and GN (12.96)
46.	MQTL3A.2 (1.38–7.47) [1.42–7.42]	IWB13817/3960111 (45.02–45.38)	12 (4.65)	GN, GY, PH, and GWei (16.24)
47.	MQTL3A.3 (19.34-107.32) [62.33–64.33]	XPacgMctg13/Xwmc532 (52.56–54.93)	7 (4.37)	GN, SRTs, DTH, PH, and GMRTs (15.26)

GY grain yield, Gwei grain weight, GN grain number, GMRTs grain morphology related traits, SRTs spike related traits, BY biomass yield, HI harvest index, DTH days to heading, DTF days to flowering, DTM days to maturity, GFD grain filling duartion, EPS earliness per se, PH plant height, TN tiller number, Avg. Average, [a]'Breeder's QTLs (had high PVE values, LOD scores, and reduced CI), [b]MQTL peak region was not calculated for the MQTLs which had ≤ 2 Mb physical interval.

Sr. no.	MQTL name (Total physical interval in Mb) [MQTL peak region]	Flanking markers (CI, in cM)	Initial QTLs (avg. LOD score)	Traits (avg. PVE)
48.	MQTL3A.4 (22.43–23.3)	AX-111488679/Xbcd372 (68.17–68.73)	21 (5.53)	SRTs, GWei, GMRTs, PH, GY, GN, HI, DTM and DTH (17.73)
49.	MQTL3A.5 (21.22–30.56) [24.89–26.89]	wPt-440/wPt-731120 (71.86–72.41)	29 (5.38)	DTH, GY, GMRTs, PH, DTF, SRTs, GWei, GN, and HI (17.88)
50.	MQTL3A.6 (21.22–26.14) [22.68–24.68]	Xfba167.1/P41/M41-4 (78.1-79.66)	18 (4.92)	GMRTs, HI, PH, SRTs, GY, GN, DTH, and GWei (16.98)
51.	MQTL3A.7 (17.6-19.61)	AX-110954980/Xmwg22 (89.7-90.45)	24 (5.83)	GWei, SRTs, GN, DTM, GY, DTH, and GMRTs (17.87)
52.	MQTL3A.8 (715.19-720.47) [716.83-718.83]	D-4261525/IWB34397 (115.44-115.65)	8 (7.61)	GN, SRTs, and PH (16.16)
53.	MQTL3B.1 (22.86–24.01)	IWB65330/IWB23552 (82.91–83.3)	11 (6.69)	GWei, GMRTs, GN, SRTs, and PH (11.71)
54.	MQTL3B.2 (23.78–28.11) [24.94–26.94]	AX-95189982/AX-94802037 (90.02–91.06)	3 (5.96)	SRTs, HI, and GWei (12.01)

GY grain yield, Gwei grain weight, GN grain number, GMRTs grain morphology related traits, SRTs spike related traits, BY biomass yield, HI harvest index, DTH days to heading, DTF days to flowering, DTM days to maturity, GFD grain filling duartion, EPS earliness per se, PH plant height, TN tiller number, Avg. Average, [a]'Breeder's QTLs (had high PVE values, LOD scores, and reduced CI), [b]MQTL peak region was not calculated for the MQTLs which had ≤ 2 Mb physical interval.

Sr. no.	MQTL name (Total physical interval in Mb) [MQTL peak region]	Flanking markers (CI, in cM)	Initial QTLs (avg. LOD score)	Traits (avg. PVE)
55.	MQTL3B.3 (24.94–28.11) [25.52–27.52]	XPaccMcga6/IWB5714 (96.31–98.38)	42 (5.81)	GN, GMRTs, SRTs, GY, BY, PH, GN, GWei, DTF, DTH, and TN (17.10)
56.	MQTL3B.4 (31.81–34.6) [32.20–34.20]	S-1090569/D-3946304 (105.69-106.47)	14 (7.47)	PH, GN, GWei, SRTs, GMRTs, BY, DTF, and GY (15.74)
57.	MQTL3B.5 (23.36–31.81) [26.58-28.589]	D-3941476/D-3532755 (115.05-115.25)	8 (5.44)	BY, GMRTs, GY, GN, and GWei (15.79)
58.	MQTL3B.6 (23.72–24.94)	D-4329780/AX-94925740 (119.94-123.07)	4 (5.91)	GY, SRTs, and GMRTs (12.99)
59.	MQTL3B.7 (749.14-769.47) [758.30-760.30]	D-1370637/D-1216223 (148.23-148.57)	6 (6.76)	GWei, GY, TN, DTH, and SRTs (14.07)
60.	[a]MQTL3D.1 (13.43–13.97)	IWB17932/wPt-1336 (1.29–2.23)	5 (62.67)	GWei, GMRTs and PH (49.16)
61.	MQTL3D.2 (7.32–9.78)	AX-109300980/Xwmc11 (17.05–22.9)	4 (4.02)	GWei, DTH, and GN (13.94)
62.	MQTL3D.3 (14.41–21.83) [17.11–19.11]	Xcfd4.1/Xgwm1243 (35.65–37.62)	2 (4.11)	SRTs and GN (12.71)
63.	MQTL3D.4 (24.79–32.47) [27.62–29.62]	AX-110430652/AX-109688927 (50.89–51.79)	2 (5.17)	SRTs and TN (14.55)

GY grain yield, Gwei grain weight, GN grain number, GMRTs grain morphology related traits, SRTs spike related traits, BY biomass yield, HI harvest index, DTH days to heading, DTF days to flowering, DTM days to maturity, GFD grain filling duartion, EPS earliness per se, PH plant height, TN tiller number, Avg. Average, [a]'Breeder's QTLs (had high PVE values, LOD scores, and reduced CI), [b]MQTL peak region was not calculated for the MQTLs which had ≤ 2 Mb physical interval.

Sr. no.	MQTL name (Total physical interval in Mb) [MQTL peak region]	Flanking markers (CI, in cM)	Initial QTLs (avg. LOD score)	Traits (avg. PVE)
64.	MQTL3D.5 (24.79–32.47) [27.62–29.62]	AX-110430652/AX-109688927 (54.43–54.72)	5 (4.82)	GWei, GN, and SRTs (23.01)
65.	MQTL3D.6 (42.3-61.23) [50.76–52.76]	AX-111337684/Xwmc529 (58.69–59.45)	2 (7.04)	PH and SRTs (11.62)
66.	MQTL3D.7 (75.67–92.38) [83.021–85.02]	TaCKX6a02-D1/CKX3D/AX-109337862 (68.35–69.39)	4 (4.07)	SRTs, GN, PH and HI (10.89)
67.	MQTL3D.8 (160.7-304.4) [231.55-233.55]	IWB54462/IWB65811 (81.61–83.75)	4 (11.40)	TN, SRTs, and GN (14.13)
68.	MQTL3D.9 (548.57-562.35) [554.46-556.46]	IWB66928/IWB30266 (117.71-119.57)	8 (3.07)	DTF, GY, GFD, SRTs, and TN (14.08)
69.	MQTL4A.1 (3.88–7.16) [4.51–6.51]	Xbcd1975b/XPaggMcgt9 (73.68–74.49)	18 (12.85)	GN, GMRTs, SRTs, DTH, GWei, and DTM (13.58)
70.	[a]MQTL4A.2 (24.76–27.67)	IWB73976/IWB39336 (138..6-138.91)	6 (15.25)	SRTs and GWei (24.89)
71.	MQTL4A.3 (709.9-713.52) [710.71-712.71]	Xcdo414/D-1229623 (201.97-202.22)	10 (5.96)	SRTs, GWei, GMRTs, PH and GY (16.77)

GY grain yield, Gwei grain weight, GN grain number, GMRTs grain morphology related traits, SRTs spike related traits, BY biomass yield, HI harvest index, DTH days to heading, DTF days to flowering, DTM days to maturity, GFD grain filling duartion, EPS earliness per se, PH plant height, TN tiller number, Avg. Average, [a]'Breeder's QTLs (had high PVE values, LOD scores, and reduced CI), [b]MQTL peak region was not calculated for the MQTLs which had ≤ 2 Mb physical interval.

Sr. no.	MQTL name (Total physical interval in Mb) [MQTL peak region]	Flanking markers (CI, in cM)	Initial QTLs (avg. LOD score)	Traits (avg. PVE)
72.	MQTL4A.4 (709.9-713.52) [710.71-712.71]	D-3948044/D-4329293 (208.54–209.3)	34 (4.76)	GWei, SRTs, BY, PH, DTH, GN, GY, HI, DTM, GMRTs and TN (18.00)
73.	MQTL4A.5 (709.9-713.52) [710.71-712.71]	BobWhite_c11327_185/Xfcp532 (221.6-221.66)	23 (5.14)	GWei, GN, SRTs, GY, BY, PH, GMRTs and TN (14.81)
74.	MQTL4B.1 (0.62–7.04) [2.83–4.83]	1123959|F|0/ACT.CTC7 (23.15–23.54)	45 (15.15)	PH, GMRTs, GWei, DTM, SRTs, and GY (18.21)
75.	[a]MQTL4B.2 (4.54–11.35) [6.94–8.94]	IWB12274/IWB73905 (34.44–35.16)	8 (19.18)	GMRTs GWei, PH, and DTM (23.36)
76.	MQTL4B.3 (11.34–11.35)	IWB73905/IWB55598 (37.75–39.02)	3 (12.55)	GWei (19.49)
77.	[a]MQTL4B.4 (13.05–13.98)	IWA7266/IWB23111 (47.12–48.28)	3 (32.60)	SRTs and PH (25.60)
78.	[a]MQTL4B.5 (13.05–14.9)	wPt-9393/wPt-7062 (51.41–51.53)	22 (17.65)	GWei, SRTs, PH, GN, and DTH (22.60)

GY grain yield, Gwei grain weight, GN grain number, GMRTs grain morphology related traits, SRTs spike related traits, BY biomass yield, HI harvest index, DTH days to heading, DTF days to flowering, DTM days to maturity, GFD grain filling duartion, EPS earliness per se, PH plant height, TN tiller number, Avg. Average, [a]'Breeder's QTLs (had high PVE values, LOD scores, and reduced CI), [b]MQTL peak region was not calculated for the MQTLs which had ≤ 2 Mb physical interval.

Sr. no.	MQTL name (Total physical interval in Mb) [MQTL peak region]	Flanking markers (CI, in cM)	Initial QTLs (avg. LOD score)	Traits (avg. PVE)
79.	MQTL4B.6 (12.89–13.42)	Xksm0154/IWB63894 (56.37–56.62)	44 (7.70)	HI, GY, SRTs, GMRTs, GWei, GN, PH, and DTH (19.79)
80.	MQTL4B.7 (605.16-619.08) [611.11-613.11]	Xmag983/D-4002948 (59.28–59.63)	27 (11.51)	GWei, GN, GY, PH, and SRTs (25.02)
81.	MQTL4B.8 (413.85–662.40) [537.12-539.12]	AX-111195232/AX-111625130 (74.47–74.52)	8 (6.78)	GMRTs, SRTs, GWei and PH (16.03)
82.	MQTL4D.1 (1.88–12.77) [6.32–8.32]	AX-109230716/Xbcd1889 (7.89–10.06)	3 (6.93)	PH (13.65)
83.	[a]MQTL4D.2 (4.76–7.81) [5.28–7.28]	Xbarc359/Xwmc574 (27.64–28.96)	16 (24.40)	GMRTs, DTM, and PH (36.83)
84.	MQTL4D.3 (3.7–6.02)	IWB19937/IWB49180 (36.82–39.39)	9 (22.09)	GWei, PH, and SRTs (24.94)
85.	MQTL4D.4 (1.41–5.56) [2.48–4.48]	IWB15470/IWB18250 (56.92–60.54)	4 (6.07)	SRTs, PH, GWei and GN (14.73)
86.	[a]MQTL4D.5 (1.24–1.66)	Xcfa2173/AX-94406142 (73.15–74.29)	9 (20.92)	PH, GWei, SRTs, HI, GN, and GY (29.98)
87.	MQTL4D.6 (3.25–3.32)	AX-109726515/Xsrap11b (84.17–84.51)	8 (10.42)	GY, BY, GMRTs, PH, GN, and HI (23.65)

GY grain yield, Gwei grain weight, GN grain number, GMRTs grain morphology related traits, SRTs spike related traits, BY biomass yield, HI harvest index, DTH days to heading, DTF days to flowering, DTM days to maturity, GFD grain filling duartion, EPS earliness per se, PH plant height, TN tiller number, Avg. Average, [a]'Breeder's QTLs (had high PVE values, LOD scores, and reduced CI), [b]MQTL peak region was not calculated for the MQTLs which had ≤ 2 Mb physical interval.

Sr. no.	MQTL name (Total physical interval in Mb) [MQTL peak region]	Flanking markers (CI, in cM)	Initial QTLs (avg. LOD score)	Traits (avg. PVE)
88.	MQTL4D.7 (3.25–5.7)	AX-110418361/AX-95116773 (92.37–93.06)	5 (16.32)	GY, BY, GN, PH and GMRTs (21.24)
89.	[a]MQTL4D.8 (5.7-13.84) [8.76–10.76]	AX-95116773/IWB53820 (94.83–96.12)	20 (18.84)	GWei, PH, SRTs, DTH, GY, TN, and GN (23.90)
90.	MQTL4D.9 (7.2–7.87)	AX-110005953/Xsrap6a (102.9-104.25)	15 (4.54)	SRTs, GMRTs, PH, GY, BY, GN, and GWei (20.24)
91.	MQTL4D.10 (39.6-40.83)	IWB2197/IWA6784 (125.98–126.4)	5 (4.83)	GY, BY, and SRTs (15.73)
92.	MQTL5A.1 (11.05–31.45) [20.25–22.25]	IWB586/IWB25701.2 (61.41–62.08)	19 (12.32)	SRTs, GMRTs, GWei, GY, and PH (15.84)
93.	MQTL5A.2 (10.84–17.53) [13.18–15.18]	AX-109887759/5AS_1513496_11975 (110.97-111.43)	71 (7.73)	GMRTs, SRTs, TN, PH, GN, DTH, GWei, GY, DTM, and DTF (18.35)
94.	[a]MQTL5A.3 (11.05–40.37) [206.38-208.38]	Xgpw2249a/wPt-9887 (135.05-135.84)	23 (17.65)	PH, DTH, SRTs, HI, GY, GMRTs, DTF, and TN (21.24)

GY grain yield, Gwei grain weight, GN grain number, GMRTs grain morphology related traits, SRTs spike related traits, BY biomass yield, HI harvest index, DTH days to heading, DTF days to flowering, DTM days to maturity, GFD grain filling duartion, EPS earliness per se, PH plant height, TN tiller number, Avg. Average, [a]'Breeder's QTLs (had high PVE values, LOD scores, and reduced CI), [b]MQTL peak region was not calculated for the MQTLs which had ≤ 2 Mb physical interval.

Sr. no.	MQTL name (Total physical interval in Mb) [MQTL peak region]	Flanking markers (CI, in cM)	Initial QTLs (avg. LOD score)	Traits (avg. PVE)
95.	MQTL5A.4 (350.27-451.48) [399.87-401.87]	D-1109368/S-988173 (175.47-176.15)	9 (10.71)	GWei, SRTs, DTH, GMRTs and PH (24.88)
96.	MQTL5B.1 (68.9-126.3) [96.60–98.60]	Kasp_5B6/Xmag4281 (34.55–36.61)	12 (8.87)	SRTs, DTH, GMRTs GWei, GY, GN, and TN (14.41)
97.	MQTL5B.2 (8.92–8.93)	AX-95249061/Xcdo665b (82.1-82.48)	10 (8.52)	SRTs, GWei, DTF, GMRTs, PH, and GN (17.64)
98.	[a]MQTL5B.3 (8.34–10.17)	AX-95107977/AX-95107977 (87.1-87.86)	4 (22.72)	SRTs, DTH and DTM (21.43)
99.	MQTL5B.4 (10.17–12.32)	Xapr1.5.4/AX-95259005 (93.73–93.92)	4 (7.55)	GMRTs, SRTs, HI and GN (13.12)
100.	MQTL5B.5 (12.32–13.73)	XPacgMcgg12/AX-94972602 (97.88–98.45)	18 (6.82)	GMRTs, GY, GWei, BY, PH, and SRTs (13.27)
101.	MQTL5B.6 (12.32–13.35)	CTCG.CAT7/D-1100080 (100.64-103.25)	5 (14.25)	DTH, GMRTs, GWei and SRTs (14.39)
102.	MQTL5B.7 (13.73–15.05)	wPt-3439c/S-1076657 (106.5-107.32)	22 (7.75)	GWei, GMRTs, DTH, TN, DTF, SRTs, and GY (15.89)

GY grain yield, Gwei grain weight, GN grain number, GMRTs grain morphology related traits, SRTs spike related traits, BY biomass yield, HI harvest index, DTH days to heading, DTF days to flowering, DTM days to maturity, GFD grain filling duartion, EPS earliness per se, PH plant height, TN tiller number, Avg. Average, [a]'Breeder's QTLs (had high PVE values, LOD scores, and reduced CI), [b]MQTL peak region was not calculated for the MQTLs which had ≤ 2 Mb physical interval.

Sr. no.	MQTL name (Total physical interval in Mb) [MQTL peak region]	Flanking markers (CI, in cM)	Initial QTLs (avg. LOD score)	Traits (avg. PVE)
103.	MQTL5B.8 (20.37–37.62) [27.14–31.14]	Xfba232a/IWB56889 (134.04-134.31)	5 (8.53)	GMRTs, SRTs, BY, and GY (16.45)
104.	MQTL5D.1 (469.52-505.13) [486.32-488.32]	S-1045585/AX-109826869 (48.72–49.95)	7 (4.92)	GWei, TN, SRTs, and GY (12.79)
105.	MQTL5D.2 (41.82–44.09)	D-1237279/AX-108840042 (76.31–79.54)	3 (11.48)	DTH, GMRTs and GWei (14.76)
106.	MQTL5D.3 (335.87-500.68) [417.27-419.27]	AX-111733701/IWB29163 (93.16–95.14)	11 (6.25)	PH, SRTs, GWei, GMRTs and DTH (20.41)
107.	MQTL5D.4 (547.71-552.68)	AX-109314990/AX-111075490 (104.66-105.21)	16 (5.85)	GY, DTF, TN, SRTs, GWei and GN (15.83)
108.	MQTL5D.5 (232.12-408.82) [319.46-321.46]	AX-111758247/AX-94469027 (115.2-118.51)	5 (6.16)	SRTs and GWei (14.46)
109.	MQTL6A.1 (555.67-563.51) [558.585–560.58]	D-3936250/GENE-4011_91 (98.34–98.8)	39 (7.39)	GN, SRTs, TN, GWei, GMRTs, PH, GY, and DTH (14.77)
110.	MQTL6A.2 (25.64-555.67) [288.65-292.65]	P78/M87.7/wPt-3191a (108.95-109.74)	27 (6.44)	GWei, PH, SRTs, GMRTs, GN, HI, GY, and DTH (17.21)

GY grain yield, Gwei grain weight, GN grain number, GMRTs grain morphology related traits, SRTs spike related traits, BY biomass yield, HI harvest index, DTH days to heading, DTF days to flowering, DTM days to maturity, GFD grain filling duartion, EPS earliness per se, PH plant height, TN tiller number, Avg. Average, [a]'Breeder's QTLs (had high PVE values, LOD scores, and reduced CI), [b]MQTL peak region was not calculated for the MQTLs which had ≤ 2 Mb physical interval.

Sr. no.	MQTL name (Total physical interval in Mb) [MQTL peak region]	Flanking markers (CI, in cM)	Initial QTLs (avg. LOD score)	Traits (avg. PVE)
111.	MQTL6A.3 (12.39–43.28) [26.83–28.83]	D-1124675/D-1076799 (150.19-151.48)	5 (6.96)	GN, SRTs, GMRTs and DTH (14.45)
112.	MQTL6B.1 (506.03-617.06) [560.54-562.541]	XPaagMctg3/AGC.TGC3 (45.07–47.28)	4 (4.81)	GWei, DTH, DTM, and SRTs (14.25)
113.	MQTL6B.2 (3.02-10) [5.50–7.50]	ACT.CAT2/P8966.1 (99.38-100.75)	10 (4.46)	DTF, GMRTs, SRTs, GY, DTH, DTF and GWei (20.73)
114.	MQTL6B.3 (3.02-10) [5.50-7.509]	P8444.1/Xksm45 (105.2-107.05)	4 (6.40)	GMRTs, GWei, and PH (12.12)
115.	MQTL6B.4 (2.17-10) [5.08–7.08]	D-379317/AGG.CAG5 (112.61-115.42)	8 (5.61)	GY, GMRTs, DTH, GWei, HI, and SRTs (13.67)
116.	MQTL6B.5 (2.17-10) [5.08–7.08]	AGG.CAG5/APaagMcgc1 (116.45-116.99)	2 (12.97)	SRTs (27.48)
117.	MQTL6B.6 (2.17-10) [5.08–7.08]	AGG.CAG5/APaagMcgc1 (118.99-119.39)	2 (15.51)	GWei and PH (14.41)
118.	MQTL6B.7 (10-11.28)	AGG.CAG5/IWB67309.2 (121.38-121.71)	3 (8.80)	GMRTs, SRTs and GWei (16.73)
119.	MQTL6B.8 (10.75–11.29)	IWB67309.2/AX-94465053 (123.2-123.74)	13 (6.44)	GMRTs, SRTs, TN, GWei, PH, and DTM (14.69)

GY grain yield, Gwei grain weight, GN grain number, GMRTs grain morphology related traits, SRTs spike related traits, BY biomass yield, HI harvest index, DTH days to heading, DTF days to flowering, DTM days to maturity, GFD grain filling duartion, EPS earliness per se, PH plant height, TN tiller number, Avg. Average, [a]'Breeder's QTLs (had high PVE values, LOD scores, and reduced CI), [b]MQTL peak region was not calculated for the MQTLs which had ≤ 2 Mb physical interval.

Sr. no.	MQTL name (Total physical interval in Mb) [MQTL peak region]	Flanking markers (CI, in cM)	Initial QTLs (avg. LOD score)	Traits (avg. PVE)
120.	MQTL6B.9 (3.88–11.43) [6.65–8.65]	Xgdm136/IWA860 (128.28-128.56)	14 (12.26)	GMRTs, GN, GY, GWei, SRTs, and DTH (19.03)
121.	MQTL6B.10 (11.43-673.36) [339.39-345.39]	IWB60027/XPaggMcat5 (151.56-202.28)	2 (5.45)	SRTs and GMRTs (11.05)
122.	MQTL6D.1 (409.34-471.01) [466.737–468.73]	IWB59376.2/IWB36391 (0-2.78)	2 (5.29)	GWei and SRTs (11.00)
123.	MQTL6D.2 (432.87-434.71)	S-3953435/1127306| F| 0 (8.62–11.11)	3 (5.03)	SRTs (15.90)
124.	MQTL6D.3 (434.71-436.71)	AX-111501437/6DL_3297209_3422 (13.58–14.37)	2 (3.04)	SRTs (12.13)
125.	MQTL6D.4 (447.8-470.32) [458.05-460.05]	AX-94493103/IWA1967.2 (31.81–35.57)	8 (4.02)	BY, GY, PH, GN, SRTs, and TN (14.58)
126.	[a]MQTL6D.5 (469.25-469.37)	IWA6361/Xcfd213b (42.88–43.58)	3 (14.05)	GWei and GY (20.73)
127.	MQTL6D.6 (465.21-468.84) [466.025–468.02]	Xwmc773/AX-109007320 (62.74–63.7)	4 (5.90)	GY, GWei, SRTs and PH (12.78)
128.	MQTL7A.1 (3.21–8.26) [4.73–6.73]	IWB7950/XgbxG564a (108.08-109.57)	39 (5.98)	DTH, GWei, DTF, DTM, TN, SRTs, GMRTs, GN, and HI (14.52)

GY grain yield, Gwei grain weight, GN grain number, GMRTs grain morphology related traits, SRTs spike related traits, BY biomass yield, HI harvest index, DTH days to heading, DTF days to flowering, DTM days to maturity, GFD grain filling duartion, EPS earliness per se, PH plant height, TN tiller number, Avg. Average, [a]'Breeder's QTLs (had high PVE values, LOD scores, and reduced CI), [b]MQTL peak region was not calculated for the MQTLs which had ≤ 2 Mb physical interval.

Sr. no.	MQTL name (Total physical interval in Mb) [MQTL peak region]	Flanking markers (CI, in cM)	Initial QTLs (avg. LOD score)	Traits (avg. PVE)
129.	MQTL7A.2 (4.06–4.63)	7AS_4114596_204/7AS_4045514_1450 (125.78-126.93)	18 (10.63)	HI, GY, DTH, SRTs, GMRTs, GWei, TN, GN, and PH (19.45)
130.	MQTL7A.3 (44.45–47.03) [43.74–47.74]	IWB207/7AS_4183259_787 (155.23-155.25)	31 (7.21)	DTF, TN, SRTs, GY, BY, GN, GWei, GMRTs and PH (19.32)
131.	MQTL7B.1 (3.7–6.37) [4.03–6.03]	CTCG.CAT2/AGT.CAGT6 (38.69–40.06)	24 (7.42)	DTH, DTM, GY, GWei, PH, SRTs, GN, HI and BY (19.33)
132.	MQTL7B.2 (18.1-44.62) [30.36-32.365]	AX-108740562/S13M23.195 (57.37–58.16)	32 (4.02)	SRTs, DTM, BY, GWei, DTH, GMRTs, DTF, BY, PH, GN and HI (16.13)
133.	MQTL7B.3 (684.43-686.14)	S-1130473/wPt-4673 (68.78–69.63)	8 (6.58)	DTF, HI, GN, GY and DTF (19.86)
134.	MQTL7B.4 (237.49-304.53) [270–272]	D-3021954/Xpsr687 (76.76–77.69)	7 (7.59)	GN, DTM, GMRTs, GY, and SRTs (16.75)
135.	MQTL7B.5 (535.26–559.3) [545.275–549.27]	Xwmc707/Xfbb258 (106.11-107.89)	8 (5.84)	GMRTs, SRTs, GWei, and TN (18.73)

GY grain yield, Gwei grain weight, GN grain number, GMRTs grain morphology related traits, SRTs spike related traits, BY biomass yield, HI harvest index, DTH days to heading, DTF days to flowering, DTM days to maturity, GFD grain filling duartion, EPS earliness per se, PH plant height, TN tiller number, Avg. Average, [a]'Breeder's QTLs (had high PVE values, LOD scores, and reduced CI), [b]MQTL peak region was not calculated for the MQTLs which had ≤ 2 Mb physical interval.

Sr. no.	MQTL name (Total physical interval in Mb) [MQTL peak region]	Flanking markers (CI, in cM)	Initial QTLs (avg. LOD score)	Traits (avg. PVE)
136.	MQTL7B.6 (711.72–713.1)	IWB68803/XPaagMcgc11 (236.54-236.79)	2 (5.39)	GY and BY (10.68)
137.	MQTL7D.1 (13.6-22.53) [17.06–19.06]	IWB517/Xgwm735b (7.29–11.22)	4 (4.38)	GFD, PH, GY, and GWei (15.19)
138.	MQTL7D.2 (53.64–76.62) [64.13–66.13]	Xswes940.3/IWB17494 (75.17–76.77)	15 (8.85)	SRTs, GY, BY, HI, DTM, DTF, GMRTs, GWei, and PH (19.49)
139.	MQTL7D.3 (501.09-526.45) [511.76-515.76]	IWB50283/IWB12642.2 (82.59–85.56)	13 (9.00)	GMRTs, SRTs, GN, GY, and DTM (21.32)
140.	MQTL7D.4 (91.01-100.28) [94.64–96.64]	D-2247427/Xwmc42 (96.65-100.15)	8 (4.58)	GMRTs and SRTs (44.55)
141.	MQTL7D.5 (562.45-588.55) [574.49-576.49]	Xpsp3123/Xswes558.1 (139.15-143.76)	6 (3.77)	GMRTs and DTM (20.70)

GY grain yield, Gwei grain weight, GN grain number, GMRTs grain morphology related traits, SRTs spike related traits, BY biomass yield, HI harvest index, DTH days to heading, DTF days to flowering, DTM days to maturity, GFD grain filling duartion, EPS earliness per se, PH plant height, TN tiller number, Avg. Average, [a]'Breeder's QTLs (had high PVE values, LOD scores, and reduced CI), [b]MQTL peak region was not calculated for the MQTLs which had ≤ 2 Mb physical interval.

Some MQTLs also harboured known genes, such as (i) MQTL5A.3, MQTL5B.3, and MQTL5B.7 included *Vrn* loci; (ii) MQTL2D.6 and MQTL2A.1 harboured *Ppd* loci; (iii) MQTL4B.5 and MQTL4B.6 included *Rht1*, and (iv) MQTL4D.5 harboured *Rht2* (Table S5). Each identified MQTL explained a large proportion of the phenotypic variation ranging from 10.68–49.16% for the different traits, whereas LOD scores for individual MQTLs ranged from 3.04 to 62.67. Generally, each MQTL was found to be associated with at least 2 different yield-related traits (Table 1). Among the 141 MQTLs, 102 MQTLs included QTLs of GWei. Similarly, 118 and 88 MQTLs contained QTLs of SRTs and GMRTs, respectively. A total of 70 MQTLs were directly associated with GY, with 60 MQTLs included QTLs of GY and GWei, 45 MQTLs contained QTLs of GY and GN, 51 MQTLs contained QTLs of GY and GMRTs, 61 MQTLs contained QTLs of GY and SRTs, 44 MQTLs contained QTLs of GY and PH, 37 MQTLs contained QTLs of GY, GWei, and GN, and 10 MQTLs

contained QTLs for 7 major traits, which included GY, GWei, GN, GMRTs, SRTs, PH, and TN. More details are presented in Table S6.

The physical interval of the MQTLs ranged from 0.01 Mb (MQTL4B.3 and MQTL5B.2) to 661.93 Mb (MQTL6B.10) with a mean of 31.47 Mb. Chromosomes 1A, 1B, 2B, 4B, 5B, 6B and 6D also carried clustered MQTLs (Fig. 3). In some cases, genetic and physical positions differed, for instance, MQTL3D.4 and MQTL3D.5 differed in genetic positions, but occupied the same physical positions. Similarly, MQTL6B.4, MQTL6B.5 and MQTL6B.6 with different genetic locations, had the same physical positions (2.17–10 Mb) on chromosome 6B (Table 1). Five clusters of MQTLs (with 362 initial QTLs), one each located on chromosomes 1B, 3B, 4D, 5B, and 6B included QTLs for almost all the traits. Details regarding these MQTLs and their associated markers along with individual traits are presented in Table 1.

Ortho-mqtls In Barley, Rice And Maize

For identification of ortho-MQTLs, 27 stable and robust wheat MQTLs were selected, each based on > 20 initial QTLs. As many as 24 corresponding MQTLs (or ortho-MQTLs) of these wheat MQTLs were identified in rice, barley, and maize genomes [5 ortho-MQTLs identified in maize; 11 in rice and maize, 2 in maize and barley, and 6 in all the three cereals (Table 2, Fig. 4)]; ortho-MQTLs for three wheat MQTLs were not available in any other cereal studied. The chromosomes of other cereals carrying the ortho-MQTLs were as follows: all the 10 maize chromosomes; 5 barley chromosomes, namely 2H, 4H, 5H, and 7H each with one ortho-MQTL; 9 of the 12 rice chromosomes (ranging from 1 ortho MQTL on chromosome 2 to 8 on chromosome 4. The remaining three rice chromosomes, namely chromosomes 1, 9, and 10 carried no ortho-MQTL. The number of MQTLs involved in an individual ortho-MQTL region ranged from 1 to 16 MQTLs (for more details see Table 2 and Table S8).

Table 2
Ortho-MQTLs in barley, rice and maize based on the syntenic region with wheat MQTLs

Sr. no.	Ortho-MQTL	Wheat MQTL (genomic position in Mb)	initial MQTL name (chr., genomic position in Mb)	Reference
1.	ortho-MQTL1A.8	MQTL1A.8 (12.11–54.01)	**Barley**: Nil **Rice**: MQTL-GW17 (5, 0.94–1.29); MQTL-HD12 (5, 21.14–27.80) **Maize**: MQTL17 (3, 95.27-107.57); MQTL36 (3, 90.04–118.17); MQTL-17 (3, 7.67–10.08); MQTL-39 (6, 0.5∼21.9)	Chen et al. (2017); Khahani et al. (2020); Wang et al. (2013, 2016);
2.	ortho-MQTL1B.5	MQTL1B.5 (17.34–17.38)	**Barley**: Nil **Rice**: Nil **Maize**: MQTL65 (6, 36.55–89.17); MQTL-41 (6, 36.56–89.15); MQTL45 (8, 73.70–95.02); MQTL84 (8, 73.22–95.76)	Chen et al. (2017); Wang et al. (2013, 2016);
3.	ortho-MQTL1B.6	MQTL1B.6 (12.81–13.24)	**Barley**: Nil **Rice**: Nil **Maize**: MQTL37 (3, 125.71–136.45); MQTL-20 (3, 86.75-136.09)	Chen et al. (2017); Wang et al. (2016)
4.	ortho-MQTL2B.3	MQTL2B.3 (28.37–29.99)	**Barley**: MQTL2H.1 (2H, 28.25–41.95) **Rice**: MQTL-HD9 (4, 2.08–6.98) **Maize**: MQTL12 (2, 154.64–174.69); MQTL24 (2, 152.70–175.81); MQTL-11 (2, 154.53-177.65); MQTL13 (2, 192.59–201.8); MQTL26 (4, 198.38–225.80); MQTL51 (4, 198.68–234.16); MQTL54 (5, 1.54–2.02); MQTL29 (5, 14.11–29.39); MQTL57 (10, 14.86–77.02); mQTL10-2 (10, 16.15–31.71); MQTL107 (10, 42.26–87.32); MQTL108 (10, 95.27–109.08); MQTL-70 (10, 85.27-102.52)	Chen et al. (2017); Khahani et al. (2019, 2020); Wang et al. (2013, 2016); Zhao et al. (2018)
5.	ortho-MQTL2B.5	MQTL2B.5 (7.11–30.49)	**Barley**: Nil **Rice**: MQTL-GW14 (4, 15.74–18.44); MQTL-PH15 (4, 20.56–23.90); MQTL-PH20 (7, 12.78–14.95); MQTL-HD15 (7, 12.78–14.95) **Maize**: MQTL11 (2, 43.63–100.18); mQTL2-2 (2, 21.78–61.80); MQTL18 (4, 9.54–14.47); mQTL5-2 (5, 135.81-168.13)	Khahani et al. (2020); Wang et al. (2013); Zhao et al. (2018)

Sr. no.	Ortho-MQTL	Wheat MQTL (genomic position in Mb)	initial MQTL name (chr., genomic position in Mb)	Reference
6.	ortho-MQTL2B.6	MQTL2B.6 (7.77–11.08)	**Barley**: Nil **Rice**: MQTL-HD9 (4, 2.08–6.98); MQTL-PH16 (4, 30.63–33.12) **Maize**: MQTL27 (2, 202.93–210.5); MQTL-14 (2, 209.83-211.52); MQTL26 (4, 198.37–225.80); MQTL51 (4, 198.68–234.17); MQTL-37 (5, 188.65-193.45); mQTL10-2 (10, 14.86–77.02)	Chen et al. (2017); Khahani et al. (2020); Wang et al. (2013, 2016); Zhao et al. (2018)
7.	ortho-MQTL2D.5	MQTL2D.5 (14.36–88.35)	**Barley**: MQTL2H.1 2H (2H, 28.25–41.95) **Rice**: Nil **Maize**: MQTL21 (2, 14.79–20.08); MQTL13 (2, 192.59–201.8); MQTL-13 (2, 199.17-205.84); MQTL46 (4, 42.17–75 – 57); MQTL-27 (4, 39.32-144.04); MQTL-69 (10, 13.06–24.61); mQTL10-2 (10, 14.86–77.02); MQTL107 (10, 42.26–87.32)	Chen et al. (2017); Khahani et al. (2019); Wang et al. (2013, 2016); Zhao et al. (2018)
8.	ortho-MQTL2D.6	MQTL2D.6 (14.90-21.06)	**Barley**: Nil **Rice**: MQTL-HD9 (4, 2.08–6.98); MQTL-GW15 (4, 23.43–24.49) **Maize**: MQTL11 (2, 43.63–100.17); MQTL23 (2, 70.33–149.74); MQTL-10 (2, 62.92-149.11); MQTL47 (4, 146.39–154.56); MQTL22 (4, 157.61–166.91); mQTL4-2 (4, 153.69–171.70); MQTL26 (4, 198.37–225.80); MQTL51 (4, 198.68–234.17); MQTL60 (5, 159.12–168.29); MQTL-36 (5, 162.84-167.47); mQTL5-2 (5, 135.81–168.13); MQTL62 (5, 202.86–207.78); MQTL-38 (5, 205.44-207.73); mQTL5-4 (5, 205.44–217.01); MQTL108 (10, 95.27–109.08); MQTL-70 (10, 85.27-102.52)	Chen et al. (2017); Khahani et al. (2020); Wang et al. (2013, 2016); Zhao et al. (2018)
9.	ortho-MQTL2D.7	MQTL2D.7 (23.42–25.04)	**Barley**: Nil **Rice**: MQTL-HD9 (4, 2.08–6.98) **Maize**: MQTL11 (2, 43.63–100.18); MQTL23 (2, 70.33–149.75); MQTL-10 (2, 62.92-149.11); MQTL-27 (4, 39.32-144.04); MQTL59 (5, 76.25–137.57)	Chen et al. (2017); Khahani et al. (2020); Wang et al. (2013, 2016)

Sr. no.	Ortho-MQTL	Wheat MQTL (genomic position in Mb)	initial MQTL name (chr., genomic position in Mb)	Reference
10.	ortho-MQTL3A.5	MQTL3A.5 (21.22–30.56)	**Barley**: Nil **Rice**: Nil **Maize**: MQTL16 (3, 11.67–29.84); MQTL83 (8, 10.85–20.83)	Wang et al. (2013, 2016)
11.	ortho-MQTL3A.7	MQTL3A.7 (17.6–19.6)	**Barley**: Nil **Rice**: Nil **Maize**: MQTL16 (3, 11.67–29.84); MQTL83 (8, 10.85–20.83)	Wang et al. (2013, 2016)
12.	ortho-MQTL4A.3	MQTL4A.3 (709.90-713.52)	**Barley**: MQTL4H.6 (4H, 632.20–641.09) **Rice**: MQTL-HD5 (3, 1.03–1.66); MQTL-TN4 (3, 7.23–9.31); MQTL-GW10 (3, 9.49–11.30); MQTL-YLD8 (3, 9.24–10.39); MQTL-GW32 (11, 5.37–8.10); MQTL-GW10 (3, 9.49–11.30); MQTL-YLD8 (3, 9.24–10.39); MQTL-GW32 (11, 5.37–8.10) **Maize**: MQTL3 (1, 34.78–44.14); MQTL6 (1, 37.47–40.20); mQTL1-3 (1, 14.79–43.06); mQTL5-2 (5, 135.81–168.13); MQTL59 (5, 76.25–137.57); MQTL-35 (5, 78.36-123.21); MQTL-66 (9, 120.2-133.6); mQTL9-3 (9, 120.65–135.91)	Chen et al. (2017); Khahani et al. (2019, 2020); Wang et al. (2013, 2016); Zhao et al. (2018)
13.	ortho-MQTL4B.1	MQTL4B.1 (0.62–7.04)	**Barley**: MQTL4H.1 (4H, 2.26–8.72) **Rice**: MQTL-TN4 (3, 7.23–9.31); MQTL-YLD19 (11, 9.06–14.95) **Maize**: MQTL1-3 (1, 14.79–43.06); mQTL1-4 (1, 148.62–191.47); mQTL1-7 (1, 258.87–290.14); MQTL29 (5, 14.11–29.39); MQTL57 (5, 16.15–31.71); mQTL1-4 (1, 148.62–191.47); mQTL1-7 (1, 258.87–290.14); MQTL29 (5, 14.11–29.39); MQTL57 (5, 16.15–31.71)	Khahani et al. (2019, 2020); Wang et al. (2013, 2016); Zhao et al. (2018)
14.	ortho-MQTL4B.5	MQTL4B.5 (13.05–14.90)	**Barley**: MQTL4H.1 (4H, 2.26–8.72) **Rice**: MQTL-GW13 (3, 30.10-30.38) **Maize**: MQTL16 (1, 214.97–224.20); mQTL1-5 (1, 209.51–, 233.37); MQTL8 (1, 256.34–262.95); mQTL1-6 (1, 257.38–269.96); MQTL29 (5, 14.11–29.39)	Khahani et al. (2019, 2020); Wang et al. (2013, 2016); Zhao et al. (2018)

Sr. no.	Ortho-MQTL	Wheat MQTL (genomic position in Mb)	initial MQTL name (chr., genomic position in Mb)	Reference
15.	ortho-MQTL4B.7	MQTL4B.7 (605.16-619.07)	**Barley**: Nil **Rice**: MQTL-PH11 (3, 1.66–2.48); MQTL-YLD19 (11, 9.06–14.95) **Maize**: MQTL6 (1, 80.18–93.0); MQTL62 (5, 202.86–207.78)	Khahani et al. (2020); Wang et al. (2013, 2016)
16.	ortho-MQTL5A.2	MQTL5A.2 (10.84–17.53)	**Barley**: Nil **Rice**: MQTL-PH28 (12, 22.45–23.06) **Maize**: MQTL26 (4, 198.37–225.80); MQTL51 (4, 198.68–234.17)	Khahani et al. (2020); Wang et al. (2013, 2016)
17.	ortho-MQTL5A.3	MQTL5A.3 (11.05-403.72)	**Barley**: MQTL5H.1 (5H, 178.37-248.57) **Rice**: Nil **Maize**: MQTL20 (4, 31.32–42.02); MQTL-27 (4, 39.32-144.04); mQTL4-1 (4, 32.24–46.45); MQTL22 (4, 157.61–166.91); MQTL48 (4, 162.92-174.12); mQTL4-2 (4, 153.69–171.70)	Chen et al. (2017); Khahani et al. (2019); Wang et al. (2013); Zhao et al. (2018)
18.	ortho-MQTL5B.7	MQTL5B.7 (13.72–15.05)	**Barley**: Nil **Rice**: MQTL-PH28 (12, 22.45–23.06) **Maize**: MQTL19 (4, 17.42–20.51)	Khahani et al. (2019, 2020); Wang et al. (2013)
19.	ortho-MQTL6A.1	MQTL6A.1 (555.67-563.51)	**Barley**: Nil **Rice**: Nil **Maize**: MQTL12 (2, 154.64–174.69); MQTL24 (2, 152.70–175.81); MQTL-11 (2, 154.53-177.65); MQTL23 (4, 173.32–181.39); MQTL49 (4, 179.77–182.35); mQTL5-4 (5, 205.44–217.01)	Chen et al. (2017); Wang et al. (2013, 2016); Zhao et al. (2018)
20.	ortho-MQTL6A.2	MQTL6A.2 (25.63–55.57)	**Barley**: Nil **Rice**: MQTL-GW6 (2, 9.56–11.75) **Maize**: MQTL51 (4, 198.68–234.17)	Khahani et al. (2020); Wang et al. (2016)

Sr. no.	Ortho-MQTL	Wheat MQTL (genomic position in Mb)	initial MQTL name (chr., genomic position in Mb)	Reference
21.	ortho-MQTL7A.1	MQTL7A.1 (3.21–8.26)	**Barley**: Nil **Rice**: MQTL-PH23 (8, 6.03–8.68); MQTL-GW25 (8, 5.80–8.15); MQTL-YLD16 (8, 11.58–17.51) **Maize**: MQTL48 (9, 26.82–28.44); mQTL9-2 (9, 26.96–105.50)	Khahani et al. (2020); Wang et al. (2013); Zhao et al. (2018)
22.	ortho-MQTL7A.3	MQTL7A.3 (44.45–47.03)	**Barley**: MQTL7H.2 (7H, 21.73–24.48) **Rice**: MQTL-HD13 (6, 1.64–2.36) **Maize**: MQTL92 (9, 5.79–9.59); MQTL47 (9, 16.66–22.11); MQTL93 (9, 17.75–23.89); MQTL-64 (9, 19.2-22.68); MQTL95 (9, 43.84–87.99); mQTL9-2 (9, 26.96–105.50)	Chen et al. (2017); Khahani et al. (2020); Wang et al. (2013, 2016); Zhao et al. (2018)
23.	ortho-MQTL7B.1	MQTL7B.1 (3.70–6.37)	**Barley**: MQTL7H.3 (7H, 34.08–38.12) **Rice**: MQTL-HD19 (8, 4.17–5.42) **Maize**: MQTL47 (9, 16.66–22.11); MQTL93 (9, 17.75–23.89); mQTL9-1 (9, 16.24–19.09)	Khahani et al. (2019, 2020); Wang et al. (2013; 2016); Zhao et al. (2018)
24.	ortho-MQTL7B.2	MQTL7B.2 (18.10-44.62)	**Barley**: Nil **Rice**: MQTL-YLD14 (6, 27.37–29.62) **Maize**: MQTL46 (9, 14.14–18.04)	Khahani et al. (2020); Wang et al. (2013)

Candidate Genes: Go Terms And Expression Patterns

Gene mining in genomic regions carrying individual MQTL allowed identification of 2,953 putative CGs; this number was reduced to 2,298 by eliminating the following: (i) duplicated genes from overlapping MQTLs; (ii) genes with no information available regarding molecular function and gene ontology (GO) terms. GO analysis gave a number of GO terms, out of which some of the crucial and most abundant GO terms include those involved in biological processes like protein ubiquitination, phosphorylation, oxidation-reduction processes and protein phosphorylation, etc. Likewise, important GO terms in molecular functions category included those participated in protein binding, DNA binding, ATP binding, ADP binding, heme binding, metal ion binding, oxidoreductase activity and transmembrane transporter

activity, etc. In terms of cellular components, the gene models were enriched primarily in the cell membrane and its components.

The *in-silico* expression analysis of the above 2.298 CGs, when screened for 2 TPM expression, only 1,202 CGs, qualified (highlighted with yellow in Table S9). The expression was examined in the following plant organs/tissues at specific developmental stages: grains, spikes, leaves, shoots, and roots, etc. (for some details, see Fig. 5; for details, see Table S9. These 1,202 CGs mainly belonged to the five major gene classes, including, (i) transcription factors, (ii) genes involved in metabolism and/or signalling of growth regulators- gibberellins, cytokinins and brassinosteroids, (iii) genes regulating cell division and proliferation (iv) floral regulators, and (v) genes involved in regulation of carbohydrate metabolism. The expression pattern of some CGs expressed in spikes and grains is shown in Fig. 5.

Several known wheat yield related genes were also co-localized with the wheat MQTLs (Table S11); 18 such genes were available, which included the following

TaSnRK2.3-1B (MQTL1B.7), *TaCwi-A1* (MQTL2A.2), *TaCYP78A5-2D* (MQTL2D.8), *FRIZZY PANICLE* (MQTL2D.5), *Btr1-A* (MQTL3A.3), *TaPSTOL* and *TaSnRK2.9-5A* (MQTL5A.3), *DEP1-5A* (MQTL5A.4), *TaCWI5D* (MQTL5D.4), *DEP1-5D* (MQTL5D.5), *TaSPL21-6A*, *TaGW2-6A*, *TaPRR1-6A*, and *TaBT1-6A* (MQTL6A.2), *TaBT1-6B, TaPRR1-6B*, and *TaSPL21-6B* (MQTL6B.10), and *TaGS3* (MQTL7A.1).

Wheat MQTLs with homology to known genes from other cereals

Known genes for yield and its component from other cereals including rice, barley, and maize were also used for the identification of wheat homologues in the MQTL regions; for this purpose, 48 genes from rice, 7 genes from barley and 13 genes from maize were available (Table S12). Wheat homologues for only 24 (50%) of the 48 rice genes, 3 of the 7 barley genes, and 7 of the 13 maize were identified in wheat MQTL regions. Of the 24 rice genes, as many as 12 genes (viz., *D2*, *DEP1*, *An-1*, *GW2*, *GIF1*, *qGL3*, *SMG1*, *OsLG3*, *OsALMT7*, *GS9*, *OsPK2*, and *FZP*) showed more than one homologues, while, remaining 12 genes showed only one homologue each in wheat MQTL regions (Table 3, S12). No wheat homologues in MQTL regions were available for four barley genes (*Ert-m, HvAPETALA2, HvLUX1*, and *INT-C*), while, two barley genes (*vrs4* and *COM1*) each gave more than one wheat homologues and one gene showed a single homologue (Table 3, S12). For 8 maize genes (*FASCIATED EAR2*, *ramosa2*, *ZmFrk1*, *bs1*, *BIF1*, *ZmGS3*, *KNR6*, and *vt2*), 12 wheat homologues were available in different MQTL regions. In some cases, more than one homologues were available within the same MQTL regions; the following are some examples: (i) MQTL2A.2 carried homologues of rice genes *An-1*, *GIF4*, *GW2*, and *OsPK2* (ii) MQTL2D.8 carried homologues of rice genes *D11*, *GIF1*, *OsPK2*, and *FZP* and (iii) MQTL3A.3 carried homologues of *Vrs4* (barley), *ramosa2*, *vt2* (maize) (Table 3). Overall, 33 MQTLs contained 50 wheat homologues involving 35 yield genes from other cereals (rice, barley, and maize).

Table 3
Wheat homologues of rice, barley and maize yield-related genes in MQTL regions

Sr. no.	Gene (associated trait)	Gene product	Wheat homologues (corresponding MQTL)	References
Rice				
1.	[a]*An-1* (GS, GN)	helix-loop-helix DNA binding domain protein	*TraesCS2A02G275600* (MQTL2A.2), *TraesCS2D02G274600* (MQTL2D.8)	Luo et al. (2013)
2.	[a]*Bsg1* (PH, FD, GY)	protein of unknown function (DUF640 domain containing protein)	*TraesCS6D02G361900* (MQTL6D.1)	Yan et al. (2013)
3.	[a]*D11* (GL)	cytochrome P450	*TraesCS2D02G331100* (MQTL2D.8)	Tanabe et al. (2005)
4.	[a]*D2* (GL)	cytochrome P450	*TraesCS3A02G103800* (MQTL3A.3), *TraesCS3D02G106100* (MQTL3D.6)	Hong et al. (2003)
5.	*DEP1* (PA, PE)	phosphatidylethanolamine-binding protein (PEBP) like domain protein	*DEP1-5A* (MQTL5A.4), *DEP1-5D* (MQTL5D.5)	Sun et al. (2014)
6.	*GIF1* (GF)	cell-wall invertase	*TaCwi-A1* (MQTL2A.2)	Wang et al. (2008)
7.	*GS3* (GS)	protein having 3 domains viz., transmembrane region, a TNFR/NGFR family cysteine-rich domain and a von Willebrand factor type C	*TaGS-D1, TraesCS7A02G017700* (MQTL7A.1)	Mao et al. (2010)
8.	*GW2* (GW, GWei)	RING-type protein with E3 ubiquitin ligase	*TaGW-A2* (MQTL6A.2), *TraesCS6B02G215300* (MQTL6B.10)	Song et al. (2007)
9.	*OsSPL14* (PB)	squamosa promter-binding-like transcription activator	*TraesCS5D02G273900* (MQTL5D.3, MQTL5D.5)	Miura et al. (2010)
10.	[a]*LP* (PL)	remorin, C-terminal region domain containing protein	*TraesCS5D02G244300* (MQTL5D.3, MQTL5D.5)	Liu et al. (2016)

GN grain number, GS grain size, PH plant height, FD floral development, GY grain yield, GL grain length, PA panicle architecture, PE panicle erectness, GF grain filling, GWei Grain weight, GW grain width, PB panicle branching, PL panicle length, TN tiller number, DTH days to heading, PBN primary branch number, PD panicle development, SD seed development, YA yield attributes, IA inflorescence architecture, and KRN kernel row number, [a]To our knowledge, orthologs of these genes have not yet been functionally characterized in wheat.

Sr. no.	Gene (associated trait)	Gene product	Wheat homologues (corresponding MQTL)	References
11.	[a]*PGL1* (GL, GWei)	similar to DNA-binding protein like, helix-loop-helix (bHLH) proteins	*TraesCS4B02G287900* (MQTL4B.8)	Heang et al. (2012)
12.	[a]*qGL3* (GS)	similar to serine/threonine protein phosphatase	*TraesCS5A02G030300* (MQTL5A.1), *TraesCS5B02G029100* (MQTL5B.8)	Qi et al. (2012)
13.	[a]*SMG1* (GS)	mitogen-activated protein kinase kinase 4,	*TraesCS6B02G379500* (MQTL6B.10), *TraesCS6D02G328800* (MQTL6D.1, MQTL6D.2, MQTL6D.3)	Duan et al. (2014)
14.	[a]*OsOTUB1* (TN, GN, GWei)	squamosa promoter-binding-like protein 14	*TraesCS5D02G273900* (MQTL5D.3, MQTL5D.5)	Wang et al. (2017)
15.	[a]*OsLG3* (GL)	APETALA2/ethylene-responsive element binding protein 125	*TraesCS6A02G097500* (MQTL6A.2), *TraesCS6B02G375400* (MQTL6B.10)	Yu et al. (2017)
16.	[a]*OsDHHC1* (TN)	Zinc finger, DHHC-type domain containing protein	*TraesCS6D02G379300* (MQTL6D.1)	Zhou et al. (2017)
17.	[a]*OsY37* (DTH)	protein similar to GRAB2	*TraesCS7D02G452500* (MQTL7D.5)	Mannai et al. (2017)
18.	[a]*qWS8* (PBN)	SBP-domain transcription factor	*TraesCS5D02G273900* (MQTL5D.3, MQTL5D.5)	Zhang et al. (2017)
19.	[a]*OsALMT7* (PD)	aluminum-activated malate transporter 10	*TraesCS6A02G255200* (MQTL6A.2), *TraesCS6B02G270300* (MQTL6B.10)	Heng et al. (2018)
20.	[a]*GS9* (GS)	uncharacterized protein	*TraesCS5A02G233400* (MQTL5A.4), *TraesCS5D02G240300* (MQTL5D.3, MQTL5D.5)	Zhao et al. (2018)
21.	[a]*GSN1* (GS, GN)	mitogen-activated protein kinase phosphatase	*TraesCS1A02G045300* (MQTL1A.2)	Guo et al. (2018)

GN grain number, GS grain size, PH plant height, FD floral development, GY grain yield, GL grain length, PA panicle architecture, PE panicle erectness, GF grain filling, GWei Grain weight, GW grain width, PB panicle branching, PL panicle length, TN tiller number, DTH days to heading, PBN primary branch number, PD panicle development, SD seed development, YA yield attributes, IA inflorescence architecture, and KRN kernel row number, [a]To our knowledge, orthologs of these genes have not yet been functionally characterized in wheat.

Sr. no.	Gene (associated trait)	Gene product	Wheat homologues (corresponding MQTL)	References
22.	[a] *OsPS1-F* (PH, TN)	*Oryza sativa* PHOTOSYSTEM 1-F subunit	*TraesCS5D02G422800* (MQTL5D.1, MQTL5D.3)	Ramamoorthy et al. (2018)
23.	[a] *OsPK2* (SD, GF)	protein similar to pyruvate kinase	*TraesCS2A02G240200* (MQTL2A.2), *TraesCS2D02G237900* (MQTL2D.8)	Cai et al. (2018)
24.	*FZP* (GS)	ethylene-responsive transcription factor FZP-like	*TraesCS2A02G116900* (MQTL2A.1), *TraesCS2D02G118200* (MQTL2D.5, MQTL2D.8)	Ren et al. (2018)
Barley				
25.	*HvCKX2* (YA)	cytokinin oxidase/dehydrogenase	*TraesCS1D02G237200* (MQTL1D.4)	Galuszka et al. (2004)
26.	[a] *Vrs4* (IA)	LOB domain TF HvRA2	*TraesCS3A02G093200* (MQTL3A.3), *TraesCS3D02G093500* (MQTL3D.6)	Koppolu et al. (2013)
27.	[a] *COM1* (IA)	AP2/ERF transcription factor	*TraesCS5A02G207300* (MQTL5A.4), *TraesCS5D02G213400* (MQTL5D.3)	Poursarebani et al. (2020)
Maize				
28.	[a] *FASCIATED EAR2* (KRN)	leucine-rich repeat receptor-like protein	*TraesCS6A02G214600* (MQTL6A.2), *TraesCS6B02G244700* (MQTL6B.10)	Bommert et al. (2013)
29.	[a] *ramosa2* (IA)	LOB Domain Protein	*TraesCS3A02G093200* (MQTL3A.3), TraesCS3D02G093500 (MQTL3D.6)	Bortiri et al. (2006)
30.	[a] *ZmFrk1* (GD)	fructokinase	*TraesCS5D02G293800* (MQTL5D.3)	Zhang et al. (2003)

GN grain number, GS grain size, PH plant height, FD floral development, GY grain yield, GL grain length, PA panicle architecture, PE panicle erectness, GF grain filling, GWei Grain weight, GW grain width, PB panicle branching, PL panicle length, TN tiller number, DTH days to heading, PBN primary branch number, PD panicle development, SD seed development, YA yield attributes, IA inflorescence architecture, and KRN kernel row number, [a]To our knowledge, orthologs of these genes have not yet been functionally characterized in wheat.

Sr. no.	Gene (associated trait)	Gene product	Wheat homologues (corresponding MQTL)	References
31.	[a]*bs1* (IA)	a putative ERF transcription factor	*TraesCS2A02G116900* (MQTL2A.1), *TraesCS2D02G118200* (MQTL2D.8)	Chuck et al. (2002)
32.	[a]*BIF1* (IA)	AUXIN/INDOLE-3-ACETIC ACID (Aux/IAA) protein	*TraesCS5A02G058600* (MQTL5A.3)	Galli et al. (2015)
33.	*ZmGS3* (SD)	protein with 198 amino acids	*TraesCS7A02G017700* (MQTL7A.1), *TraesCS6B02G136400* (MQTL6B.10)	Li et al. (2010)
34.	*ZmVT2* (IA)	grass-specific tryptophan aminotransferase	*TraesCS3A02G093000* (MQTL3A.3)	Phillips et al. (2011)
35.	[a]*KNR6* (KRN)	a serine/threonine protein kinase	*TraesCS7D02G451300* (MQTL7D.5)	Jia et al. (2020)

GN grain number, GS grain size, PH plant height, FD floral development, GY grain yield, GL grain length, PA panicle architecture, PE panicle erectness, GF grain filling, GWei Grain weight, GW grain width, PB panicle branching, PL panicle length, TN tiller number, DTH days to heading, PBN primary branch number, PD panicle development, SD seed development, YA yield attributes, IA inflorescence architecture, and KRN kernel row number, [a]To our knowledge, orthologs of these genes have not yet been functionally characterized in wheat.

Discussion

During the past two decades, starting with the first QTL studies on yield-related traits published by Araki et al. (1999) and Kato et al. (1999), a large number of studies have been conducted on QTL mapping for grain yield and its component traits in wheat (Table S1). The studies involving the development of MQTLs were largely motivated by the fact that only a small fraction of QTLs identified by interval mapping are major QTLs, and the majority of QTLs are each associated with a large CI, with flanking markers often located away from the QTLs, thus making these QTLs not very useful for plant breeding. Also, QTLs identified using one bi-parental population may not be effective for a breeding programme involving other parents, without prior validation, unless the markers are functional markers located within the QTLs. These problems can be largely overcome through the development of MQTLs, which are robust with a reduced CI, thus increasing the utility of these MQTLs not only in crop improvement programmes, but also for basic studies involving cloning and characterizing QTLs/genes for the traits of interest.

Meta-QTL analysis has been conducted for a variety of traits in all the major crops. As mentioned earlier, in wheat, meta-QTL analysis has been conducted for several traits including yield (Griffiths et al. 2009; Gegas et al. 2010; Zhang et al. 2010; Griffiths et al. 2012; Quraishi et al. 2017; Bilgrami et al. 2020; Liu et al. 2020), but the information on MQTLs soon becomes out-of-date. This is because, a large number of

studies on QTL analysis for yield traits in wheat are regularly conducted, thus creating a need for conducting further studies on MQTLs periodically to obtain improved MQTLs. The present study is one such attempt, conducted to improve upon MQTLs reported so far for grain yield and associated traits in wheat. The latest studies on meta-QTL analysis for yield in hexaploid and durum wheat were conducted by Yang et al. (2021) and Maccaferri et al. (2019) respectively, where 2,230 QTLs in hexaploid wheat (collected from 119 studies) and 1,162 QTLs for durum wheat were utilized leading to identification 145 MQTLs for hexaploid wheat and 71 MQTL for durum wheat. In contrast to this, the number of available QTLs that we collected were 8,998, of which 2,852 major QTLs were used for identification of as many as 141 MQTLs suggesting that the present study is so far the most comprehensive study for identification of MQTLs in wheat. Flow diagram of meta-QTL analysis applied in this study is shown in Fig. 6.

The results of the present study along with earlier studies suggest that the precision of the results of the meta-analysis depend at least partly on the number of QTLs available for meta-QTL analysis (Quraishi et al. 2017; Soriano et al., 2021). It may be recalled that in the present study, 63 MQTLs (44.68%) were each based on ≥ 10 initial QTLs, 16 of them based on >30 QTLs; these frequencies of QTLs per MQTL are higher than each of the earlier studies (Quraishi et al. 2017; Liu et al. 2020). Also, 24 MQTLs in the present study had their genetic positions almost overlapping those occupied by MQTLs reported in two recent studies (Bilgrami et al. 2020; Liu et al. 2020) so that these MQTLs can be used in future studies with a higher level of confidence (Table S5). On a critical evaluation of these 24 MQTLs, we selected 15 MQTLs each involving at least 10 initial QTLs; these MQTLs can be used with a higher level of confidence for molecular breeding and future studies for cloning and characterization of QTLs/genes (Table S5).

Another benefit of meta-QTL analysis is that it effectively reduces the CI of QTLs by aggregating QTL information from different genetic backgrounds, making it easier to transfer and consolidate important QTL regions in wheat breeding and improving CG prediction (Liu et al. 2020). The CI of MQTLs was 8.87 times narrower than that of initial QTLs, which is much better than the 2.44 times (12.7 cM/5.2 cM) achieved by Liu et al. (2020) and 2.92 times achieved by Yang et al. (2021). On the other extreme, one MQTL (MQTL6B.10) identified in the present study, had a CI of 50.72 cM, which was longer than even the CIs of the two corresponding participating QTLs (Table 1). No suitable explanation for this is available at present. We may need to investigate this MQTL further to explain this anomalous situation. One possible explanation is that the genomic regions involved may represent a region of unusual and abnormal recombination frequencies.

Breeder's QTLs

In the present study, 13 MQTLs were described as "Breeder's QTLs" because these are believed to important for wheat breeders since each had a small CI (<2 cM) and explained a significant proportion of phenotypic variation (ranging from 20.73 to 49.16 %) associated with high LOD values (ranging from 14.05 to 62.67) (Table 1). Of these breeder's QTLs, MQTL3D.1 had a PVE value of 49.16% and therefore can be considered to be a mega breeder QTL for three traits (GWei, GMRTs, and PH). Sequences of the markers associated with these breeder's QTLs are provided in Table S7. Availability of clusters of MQTLs,

one cluster each on seven different chromosomes, is another important feature of the present study (Fig. 3). These clusters of MQTLs may be treated as hotspots and can be utilized for breeding and future basic research with a high level of confidence.

Ortho-MQTLs for cereals

In the present study, 24 ortho-MQTLs were also identified, which should represent conserved genomic regions and therefore may be recommended for use across the cereals. The conserved nature of these ortho-MQTLs also suggests that these may be associated with some regulatory elements, each influencing many genes (Quraishi et al. 2011; Jin et al. 2015; Khahani et al. 2020). As many as six of these 24 ortho-MQTLs identified in the present study were cross-species in all four crops, revealing the high level of conservation of wheat with barley, maize, and rice. Ortho-MQTLs for three of the twenty-seven investigated wheat MQTLs (MQTL1A.5, MQTL2A.2, and MQTL3A.4) were not identified on any syntenic chromosome of the studied species this may be because, firstly, they are unique MQTLs which do not have orthologs in any of the studied species (abundance of insertions, deletions, rearrangements, and duplications occurred during the cereal evolution might have disrupted the colinearity of genes in the target regions), and secondly, their corresponding syntenic regions in rice, barley and maize have not yet been investigated for the identification of QTLs for yield and related traits. These wheat MQTLs may be investigated further as they are supposed to provide novel sources to manipulate crop yield particularly in wheat.

Of the CGs underlying these ortho-MQTLs, precise orthologous gene sets can be considered as direct potential candidates for further homology-based cloning, functional validation, or at least as a source of accurate molecular markers such as conserved orthologous set (COS) markers for use in cereal breeding programs. The success of this approach is apparent from at least two earlier studies. In wheat, an ortho-MQTL associated with nitrogen use efficiency was characterized as the conserved gene 'glutamate synthase' (*GoGAT*) (Quraishi et al. 2011). In another study, dissection of ortho-MQTLs associated with grain iron and zinc led to the identification of two genes namely *GRMZM2G178190,* and *GRMZM2G366919*. These genes were characterized as natural resistance-associated macrophage protein genes and considered to be the best candidate genes associated with grain iron and zinc in maize (Jin et al. 2015).

Candidate genes for MQTLs

In the present study, 1204 CGs of 2298 CGs had >2 TPM expression; 28 of these putative CGs had >10 TPM expression (Table 9) in different plant tissues at different times (spatio-temporal gene expression). As mentioned earlier, these putative CGs mainly belonged to five major categories of the genes which are known to be involved in controlling the grain yield and associated traits in cereals (Nadolska-Orczyk et al. 2017; Daba et al. 2020) (Table S10). In the present study, several genes/gene families with similar functions were detected repeatedly in different MQTL regions. Association of these genes/gene families with grain yield and its component traits has been reported in several studies (Ma et al. 2017; Nadolska-Orczyk et al. 2017; Gunupuru et al. 2018; Sakuma et al. 2018; Niño-González et al. 2019; Gautam et al.

2019; Daba et al. 2020; Jia et al. 2020; Li and Wei, 2020). These genes included 114 genes encoding proteins with leucine-rich repeat (LRR) domain,, 63 genes for serine/threonine-protein kinases, 33 genes for cytochrome P450 proteins, and 14 genes each for WD40/YVTN repeat-like containing proteins, UDP-glucuronosyl/UDP-glucosyltransferases, FAD/NAD(P) binding proteins, and E3 ubiquitin ligases, etc (Table S9). Moreover, some genes encoding unpredicted or uncharacterized proteins also showed significant expression in different plant tissues (Table S9). These genes deserve further investigation, to explore their possible roles in the regulation of yield and its component traits in wheat.

In some MQTL regions, we also identified several clusters of genes associated with specific gene superfamilies, which included the following: kinase-like domain superfamily, F-box-like domain superfamily, UDP-glucuronosyl/UDP-glucosyltransferase, etc. (Table S9). These gene clusters are quite common in plant genomes and are known to encode proteins involved in many enzymatic pathways in plants (Yi et al. 2007; Medema et al. 2015). These members of a gene cluster are found to be located in proximity (only a few thousand base pairs far from each other) in a small genomic region, encoding similar products or proteins, thus together sharing a generalized function.

We selected as many as 162 high confidence CGs, most of which had more than 5 TPM expressions in different tissues (Table 4). Most of these CGs showed the highest expression in the spike, spike organs and grains (including the grain tissues such as endosperm, embryo, aleurone layer, seed coat, and transfer cells) at the reproductive stage and therefore, supposed to affect SRTs, GWei, GN, and GMRTs traits; while remaining CGs showed the highest expression in the root, leaves and stem tissues at the vegetative stage and therefore believed to affect TN, HI, and BY of the plants (Table 4). An extensive survey of available literature also shows the association of these selected genes with the traits of interest in different plant species. These CGs may be further characterized and cloned and then can be exploited through biotechnological approaches such as transgenesis and gene editing. In a more recent study, it was observed that over-expression of the expansin gene in developing seeds minimizes the trade-off between grain number and grain weight, and ultimately improves the grain yield. Transgenic plants with enhanced expression of the Expansin gene yielded 12.3 % higher grain weight compared to the control, which finally translated into an 11.3% increase in grain yield under field conditions (Calderini et al.2020). In the present study, we also identified many putative CGs, including genes for Expansin proteins in some MQTL regions (Table 4, S8). In the future, the targeted transgenic approach using these potential CGs may allow improvement for grain yield in wheat. However, in some cases, where gene clusters regulate the expression of target trait, the transgenic method using a single gene may not be as effective as MAS, where flanking markers can target a much larger region encompassing all the genes of a cluster.

Table 4 High confidence CGs identified in the present study

Sr. no.	MQTL name (Total CGs identified; CGs with ≥ 2 TPM expression)	Putative Gene ID	Gene Function Description	Tissue (max. TPM value observed)
1.	MQTL1A.2 (11; 7)	*TraesCS1A02G043600*	RNA polymerase II transcription factor SIII	Spike (5.38)
		TraesCS1A02G044200	WD40/YVTN repeat-like-containing protein	Spike (4.23)
2.	MQTL1A.3 (31; 7)	*TraesCS1A02G040600*	Phosphoglucose isomerase	Leaves/shoots (6.54)
		TraesCS1A02G041300	Proteinase inhibitor I13	Leaves/shoots (10.19)
3.	MQTL1A.4 (5; 4)	*TraesCS1A02G042700*	SANT/Myb protein	Roots (8.24)
4.	MQTL1A.6 (5; 3)	*TraesCS1A02G060700*	Ribosomal biogenesis regulatory protein	Grain (6.81)
5.	MQTL1A.7 (3; 3)	*TraesCS1A02G046500*	E3 UFM1-protein ligase 1	Leaves/shoots (5.40)
		TraesCS1A02G047000	HAD-like superfamily	Leaves/shoots (5.16)
6.	MQTL1A.8 (16; 12)	*TraesCS1A02G050400*	SWEET sugar transporter	Grain (5.71)
		TraesCS1A02G050800	Defensin	Grain (10.74)
7.	MQTL1B.1 (44; 31)	*TraesCS1B02G006500*	Serine/threonine-protein kinase	Leaves/shoots (5.19)
		TraesCS1B02G008000	RNA-binding domain superfamily	Spike (5.43)
8.	MQTL1B.2 (7; 5)	*TraesCS1B02G068200*	HSP20-like chaperone	Roots (6.69)
9.	MQTL1B.3 (6; 5)	*TraesCS1B02G020700*	Serine/threonine-protein kinase	Leaves/shoots (6.56)
10.	MQTL1B.4 (30; 16)	*TraesCS1B02G026500*	Leucine-rich repeat protein	Roots (2.36)
11.	MQTL1B.5 (3; 2)	*TraesCS1B02G036500*	Calcium-dependent channel	Leaves/shoots (5.23)
12.	MQTL1B.7 (10; 8)	*TraesCS1B02G223600*	Putative NADH dehydrogenase	Roots (6.50)
		TraesCS1B02G224300	Trehalose-phosphatase	Roots (7.10)
13.	MQTL1B.8 (14;	*TraesCS1B02G261700*	GDSL lipase/esterase	Spike (5.92)

	12)	*TraesCS1B02G262000*	Oxoglutarate/iron-dependent dioxygenase	Leaves/shoots (6.99)
14.	MQTL1D.1 (5; 5)	*TraesCS1D02G003900*	Ribosomal protein L28/L24	Leaves/shoots (5.92)
15.	MQTL1D.6 (17; 3)	*TraesCS1D02G037900*	Chalcone isomerase	Leaves/shoots (5.73)
16.	MQTL2A.2 (1; 1)	*TraesCS2A02G245900*	Ribosomal protein S19	Leaves/shoots (8.60)
17.	MQTL2A.3 (19; 12)	*TraesCS2A02G089300*	Heat shock transcription factor family	Grain (5.87)
		TraesCS2A02G090000	P-loop containing nucleoside triphosphate hydrolase	Leaves/shoots (7.23)
18.	MQTL2A.4 (25; 12)	*TraesCS2A02G064300*	Zinc finger protein	Grain (5.07)
19.	MQTL2A.5 (10; 5)	*TraesCS2A02G065100*	Protein-tyrosine phosphatase	Leaves/shoots (5.60)
		TraesCS2A02G065700	Major intrinsic protein	Spike (6.78)
20.	MQTL2A.6 (24; 16)	*TraesCS2A02G071500*	Flavin monooxygenase	Grain (5.31)
21.	MQTL2A.7 (13; 10)	*TraesCS2A02G086400*	P-loop containing nucleoside triphosphate hydrolase	Roots (5.87)
22.	MQTL2B.1 (41; 17)	*TraesCS2B02G027000*	Protein kinase-like domain superfamily	Roots (5.86)
		TraesCS2B02G030700	Zinc finger protein	Spike (8.00)
23.	MQTL2B.2 (14; 10)	*TraesCS2B02G053200*	Glycoside hydrolase	Roots (5.42)
24.	MQTL2B.3 (20; 12)	*TraesCS2B02G059500*	Flowering-promoting factor 1	Leaves/shoots (5.62)
25.	MQTL2B.4 (10; 5)	*TraesCS2B02G063500*	P-loop containing nucleoside triphosphate hydrolase	Roots (5.35)
26.	MQTL2B.6 (27; 6)	*TraesCS2B02G020400*	Polycomb protein	Spike (5.95)
		TraesCS2B02G021500	Protein kinase-like domain superfamily	Roots (7.09)
27.	MQTL2B.7 (21; 4)	*TraesCS2B02G090300*	RmlC-like cupin protein	Leaves/shoots (10.75)
28.	MQTL2D.1 (29; 16)	*TraesCS2D02G006500*	Cytochrome P450 protein	Leaves/shoots (6.26)

29.	MQTL2D.2 (26; 11)	*TraesCS2D02G020900*	Photosystem II PsbI	Leaves/shoots (7.72)
		TraesCS2D02G021500	Cytochrome P450 protein	Leaves/shoots (6.17)
		TraesCS2D02G022700	Chalcone/stilbene synthase	Leaves/shoots (7.17)
30.	MQTL2D.3 (39; 14)	*TraesCS2D02G057000*	Glycoside hydrolase	Grain (10.02)
		TraesCS2D02G055000	UDP-glycosyltransferase	Leaves/shoots (6.22)
31.	MQTL2D.5 (17; 13)	*TraesCS2D02G099400*	Ethylene insensitive 3-like protein	Leaves/shoots (5.56)
		TraesCS2D02G100300	Zinc finger protein	Roots (5.10)
32.	MQTL2D.6 (24; 15)	*TraesCS2D02G047800*	Defensin	Spike (6.84)
		TraesCS2D02G049500	Chaperonin containing TCP-1	Leaves/shoots (9.27)
33.	MQTL2D.8 (8; 8)	*TraesCS2D02G250300*	P-loop containing nucleoside triphosphate hydrolase	Leaves/shoots (5.09)
		TraesCS2D02G251000	RNA-binding protein	Leaves/shoots (7.41)
34.	MQTL3A.1 (30; 22)	*TraesCS3A02G008700*	Aquaporin transporter	Spike (7.53)
		TraesCS3A02G011100	Expansin	Grain (6.67)
35.	MQTL3A.2 (18; 13)	*TraesCS3A02G003800*	Thiolase-like	Leaves/shoots (5.64)
36.	MQTL3A.3 (16; 14)	*TraesCS3A02G097100*	Sugar phosphate transporter	Spike (7.23)
		TraesCS3A02G099200	AP2/ERF	Grain (6.58)
37.	MQTL3A.4 (5; 3)	*TraesCS3A02G041800*	Tryptophan synthase	Leaves/shoots (6.30)
38.	MQTL3A.5 (26; 6)	*TraesCS3A02G049600*	Serine/threonine-protein kinase	Leaves/shoots (5.69)
39.	MQTL3A.6 (28; 13)	*TraesCS3A02G044800*	Mannose-6-phosphate isomerase	Grain (5.18)
40.	MQTL3A.7 (23; 9)	*TraesCS3A02G033900*	HSP20-like chaperone	Grain (8.26)
41.	MQTL3A.8 (19; 15)	*TraesCS3A02G490300*	Flavoprotein-like protein	Roots (6.01)
		TraesCS3A02G489700	Helix-loop-helix DNA-binding protein	Roots (5.83)

42.	MQTL3B.1 (20; 13)	*TraesCS3B02G045700*	Glycoside hydrolase family 10	Spike (5.71)
		TraesCS3B02G047300	Sugar/inositol transporter	Grain (7.63)
43.	MQTL3B.2 (34; 16)	*TraesCS3B02G050500*	Mitochondrial carriers	Leaves/shoots (5.39)
		TraesCS3B02G052600	Serine/threonine-protein kinase	Leaves/shoots (5.10)
44.	MQTL3B.4 (7; 3)	*TraesCS3B02G061700*	Photosystem I PsaL	Leaves/shoots (10.87)
45.	MQTL3B.6 (8; 2)	*TraesCS3B02G048900*	Short-chain dehydrogenase/reductase	Roots (5.17)
46.	MQTL3B.7 (4; 2)	*TraesCS3B02G517100*	RNA-binding protein	Grain (5.77)
47.	MQTL3D.1 (7; 3)	*TraesCS3D02G037600*	Ribosomal protein S10	Grain (5.87)
48.	MQTL3D.2 (46; 25)	*TraesCS3D02G024500*	Glycosyltransferase 61	Roots (6.22)
		TraesCS3D02G024700	Cytochrome P450 protein	Spike (7.69)
49.	MQTL3D.3 (27; 18)	*TraesCS3D02G046700*	HSP20-like chaperone	Grain (9.40)
50.	MQTL3D.4, & 5 (29; 16)	*TraesCS3D02G063100*	Serine/threonine-protein kinase	Leaves/shoots (5.29)
51.	MQTL3D.6 (6; 5)	*TraesCS3D02G099900*	Golgi to ER traffic protein 4	Spike (5.32)
52.	MQTL3D.7 (21; 10)	*TraesCS3D02G126500*	UDP-glucuronosyl/UDP-glucosyltransferase	Leaves/shoots (5.90)
53.	MQTL3D.9 (24; 14)	*TraesCS3D02G446700*	C2 domain superfamily	Spike (7.28)
54.	MQTL4A.1 (40; 24)	*TraesCS4A02G007400*	Thiolase-like	Grain (8.07)
		TraesCS4A02G011300	Protein of unknown function DUF538	Grain (6.21)
55.	MQTL4A.2 (28; 11)	*TraesCS4A02G035400*	AP2/ERF	Leaves/shoots (8.13)
56.	MQTL4A.3, 4 & 5 (8; 7)	*TraesCS4A02G442900*	Peroxisomal biogenesis factor 11	Grain (6.62)
57.	MQTL4B.1 (22; 6)	*TraesCS4B02G005900*	Cytochrome P450 protein	Root (6.78)
58.	MQTL4B.2 (26; 4)	*TraesCS4B02G011500*	HPT domain superfamily	Grain (9.95)
59.	MQTL4B.3 (1; 1)	*TraesCS4B02G015100*	Replication factor A protein-like	Grain (5.63)
60.	MQTL4B.4 (11;	*TraesCS4B02G017500*	RmlC-like cupin	Grain (12.86)

	11)			
61.	MQTL4B.5 (18; 8)	*TraesCS4B02G020300*	RNA-binding protein	Leaves/shoots (10.89)
62.	MQTL4B.7 (16; 14)	*TraesCS4B02G321200*	Fasciclin-like arabinogalactan protein	Leaves/shoots (5.20)
		TraesCS4B02G321300	Glycosyl transferase	Leaves/shoots (5.66)
63.	MQTL4B.8 (8; 4)	*TraesCS4B02G266700*	GRAS TF	Roots (5.23)
64.	MQTL4D.1 (46; 39)	*TraesCS4D02G014100*	WRKY TF	Leaves/shoots (6.74)
		TraesCS4D02G018100	Zinc finger protein	Grain (5.57)
65.	MQTL4D.2 (22; 9)	*TraesCS4D02G012100*	Short-chain dehydrogenase/reductase	
66.	MQTL4D.3 (29; 23)	*TraesCS4D02G008700*	Signal transduction histidine kinase	Grain (5.18)
67.	MQTL4D.4 (19; 12)	*TraesCS4D02G006100*	Glycoside hydrolase	Spike (5.04)
		TraesCS4D02G006900	Ubiquitin-like protein	Leaves/shoots (8.73)
68.	MQTL4D.5 (13; 12)	*TraesCS4D02G003100*	Major facilitator superfamily	Leaves/shoots (5.80)
69.	MQTL4D.8 (33; 25)	*TraesCS4D02G021100*	MADS-box TF	Spike (5.73)
		TraesCS4D02G022600	Homeobox-like domain superfamily	Leaves/shoots (5.55)
70.	MQTL4D.10 (16; 9)	*TraesCS4D02G064400*	WD40/YVTN repeat-like-containing protein	Grain (5.93)
		TraesCS4D02G065300	MFS transporter superfamily	Roots (5.09)
		TraesCS4D02G065600	Glycoside hydrolase family 9	Leaves/shoots (8.25)
71.	MQTL5A.1 (6; 3)	*TraesCS5A02G025900*	YABBY protein	Grain (8.69)
72.	MQTL5A.2 (15; 14)	*TraesCS5A02G019200*	Fatty acid hydroxylase	Roots (5.29)
73.	MQTL5A.3 (4; 3)	*TraesCS5A02G110200*	Pentatricopeptide repeat	Leaves/shoots (3.05)
74.	MQTL5A.4 (15; 11)	*TraesCS5A02G196400*	Ubiquitin-like domain superfamily	Leaves/shoots (7.37)
		TraesCS5A02G197000	Protein kinase-like domain superfamily	Leaves/shoots (5.41)

75.	MQTL5B.1 (10; 4)	*TraesCS5B02G080200*	Ubiquitin-like domain superfamily	Spike (11.33)
76.	MQTL5B.3 (15; 10)	*TraesCS5B02G007100*	Cytochrome P450 protein	Leaves/shoots (8.30)
		TraesCS5B02G007500	Ankyrin repeat-containing protein	Spike (5.07)
77.	MQTL5B.4 (12; 11)	*TraesCS5B02G010500*	Thiolase-like	Leaves/shoots (6.50)
		TraesCS5B02G011700	Glycoside hydrolase	Grain (7.69)
78.	MQTL5B.5 & 6 (17; 16)	*TraesCS5B02G013200*	RNA recognition motif domain	Root (11.39)
		TraesCS5B02G014400	Small GTPase	Grain (5.89)
79.	MQTL5B.7 (9; 8)	*TraesCS5B02G015000*	ATP synthase	Spike (9.12)
80.	MQTL5B.8 (12; 11)	*TraesCS5B02G028300*	Short-chain dehydrogenase/reductase	Grain (7.00)
		TraesCS5B02G029000	Regulator of K+ conductance	Leaves/shoots (6.85)
81.	MQTL5D.1 (19; 7)	*TraesCS5D02G431400*	Armadillo-like helical	Grain (7.73)
		TraesCS5D02G430000	Clathrin adaptor complex	Root (7.45)
82.	MQTL5D.2 (23; 9)	*TraesCS5D02G044100*	Subtilisin-like protease	Leaves/shoots (5.89)
83.	MQTL5D.4 (62; 35)	*TraesCS5D02G536500*	Nucleotide-diphospho-sugar transferase	Grain (6.82)
		TraesCS5D02G536100	Mitochondrial carrier	Leaves/shoots (5.45)
		TraesCS5D02G535400	Cytochrome P450 protein	Grain (8.69)
84.	MQTL5D.5 (8; 7)	*TraesCS5D02G211200*	TIFY/JAZ family	Spike (8.81)
		TraesCS5D02G211900	Ubiquitin-like domain superfamily	Roots (5.90)
85.	MQTL6A.1 (24; 15)	*TraesCS6A02G325400*	Oxoglutarate/iron-dependent dioxygenase	Spike (10.74)
		TraesCS6A02G325100	Transcription elongation factor Spt5	Spike (5.86)
		TraesCS6A02G325300	Glycoside hydrolase	Spike (6.31)
86.	MQTL6A.2 (2; 1)	*TraesCS6A02G196800*	RmlC-like jelly roll fold	Leaves/shoots (5.53)

87.	MQTL6A.3 (1; 1)	*TraesCS6A02G196600*	Glycosyltransferase	Roots (3.45)
88.	MQTL6B.1 (15; 10)	*TraesCS6B02G313100*	WD40/YVTN repeat-like-containing protein	Leaves/shoots (7.49)
		TraesCS6B02G313400	GPI-anchored protein LORELEI-like	Grain (7.52)
89.	MQTL6B.2 & 3 (33; 19)	*TraesCS6B02G012100*	GroEL-like apical domain superfamily	Leaves/shoots (6.27)
90.	MQTL6B.7 (12; 3)	*TraesCS6B02G017700*	RNA-binding protein	Grain (7.20)
91.	MQTL6B.10 (3; 2)	*TraesCS6B02G224800*	Translation elongation factor EFTu-like	Leaves/shoots (8.16)
92.	MQTL6D.1 (34; 23)	*TraesCS6D02G392100*	Glycoside hydrolase	Leaves/shoots (5.37)
		TraesCS6D02G392600	Serine/threonine-protein kinase	Leaves/shoots (5.39)
93.	MQTL6D.2 (34; 19)	*TraesCS6D02G331400*	Amino acid transporter	Leaves/shoots (7.64)
		TraesCS6D02G330800	Diacylglycerol kinase	Roots (6.05)
94.	MQTL6D.3 (19; 15)	*TraesCS6D02G334000*	Glycosyl transferase	Leaves/shoots (7.93)
		TraesCS6D02G332900	Dehydrin	Grain (10.77)
95.	MQTL6D.4 (25; 12)	*TraesCS6D02G375700*	Serine/threonine-protein kinase	Leaves/shoots (5.56)
96.	MQTL6D.5 (31; 23)	*TraesCS6D02G399500*	Leucine-rich repeat protein	Spike (5.68)
		TraesCS6D02G397800	Gibberellin regulated protein	Spike (6.03)
97.	MQTL7A.1 (39; 7)	*TraesCS7A02G014100*	Ribosomal protein L9	Grain (5.86)
		TraesCS7A02G015300	Serine-threonine/tyrosine-protein kinase	Leaves/shoots (6.75)
98.	MQTL7A.2 (8; 6)	*TraesCS7A02G009400*	Glycoside hydrolase	Grain (6.28)
		TraesCS7A02G009900	WD40/YVTN repeat-like-containing protein	Spike (5.48)
99.	MQTL7A.3 (18; 11)	*TraesCS7A02G082000*	Ribosomal protein L5	Spike (6.13)
100.	MQTL7B.1 (19; 9)	*TraesCS7B02G008400*	F-box-like domain superfamily	Leaves/shoots (5.00)
		TraesCS7B02G009200	Mitogen-activated protein kinase	Grain (5.87)
101.	MQTL7B.2 (5; 4)	*TraesCS7B02G031400*	Casparian strip membrane	Roots (5.25)

			protein	
102.	MQTL7B.5 (6; 5)	*TraesCS7B02G304500*	Glycoside hydrolase	Spike (8.54)
103.	MQTL7B.6 (15; 8)	*TraesCS7B02G450100*	Senescence/spartin-associated	Grain (6.72)
		TraesCS7B02G451200	SWEET sugar transporter	Spike (5.72)
104.	MQTL7D.1 (29; 18)	*TraesCS7D02G036600*	Sucrose synthase	Leaves/shoots (10.54)
		TraesCS7D02G036900	Cytochrome P450 protein	Leaves/shoots (5.23)
105.	MQTL7D.2 (23; l1)	*TraesCS7D02G106400*	Mitogen-activated protein kinase	Grain (6.17)
		TraesCS7D02G108900	GDSL lipase/esterase	Roots (7.38)
106.	MQTL7D.3 (17; 12)	*TraesCS7D02G398900*	Glycoside hydrolase	Spike (8.23)
107.	MQTL7D.5 (30; 18)	*TraesCS7D02G455900*	Small auxin-up RNA	Leaves/shoots (5.79)
		TraesCS7D02G456600	Ribosomal protein S13	Grain (5.82)
		TraesCS7D02G457400	SANT/Myb protein	Spike (2.96)
		TraesCS7D02G458200	Papain-like cysteine peptidase	Grain (5.73)

MQTL regions were also examined for genes already known to be associated with yield and its component traits; as many as 18 such wheat genes were identified. These genes encode a variety of proteins spanning all major pathways, for instance, cell wall invertase, sucrose non-fermenting 1-related protein kinase, E3 ubiquitin ligase, APETALA2/AP2/ERF transcription factor, cytochrome P450 protein, and phosphatidylethanolamine binding protein. Similar proteins/products are also encoded by many other CGs, identified in the present study. Therefore, these CGs can be used for further functional analysis. Some of the MQTLs also included some known genes, such as, *Vrn*, *Ppd*, and *Rht* genes, *that are widely known to regulate plant phenology, ultimately influencing the grain yield and other component traits in wheat (*Kamran et al. 2014; Gupta et al. 2020).

Wheat homologues of rice, barley and maize genes

During the present study, within the wheat MQTL regions, we also identified 50 wheat homologues of 35 genes that are known to control grain yield and related traits in rice, barley, and maize; to our knowledge, homologues of only 8 of these 35 alien genes (*GW2, GIF1, GS3, DEP1, CKX2, OsSPL14, FZP,* and *ZmVT2)* have been cloned and characterized in wheat; following are the details of these wheat homologues: *TaGW2* (Su et al. 2011), *TaCwi-A1* (Ma et al. 2012), *TaGS-D1* (Zhang et al. 2014), *DEP1* (Vavilova et al. 2017), *TaCKX* family genes(Ogonowska et al. 2019), *TaSPL14* (Cao et al. 2021),

FRIZZY PANICLE (FZP) (Dobrovolskaya et al. 2015), and *TaTAR2.1 (Shao et al. 2017), while,* remaining genes have not yet been cloned and functionally characterized in wheat, these genes include the following: (i) rice genes: *An-1, Bsg1, D11, D2, LP, PGL1, qGL3, SMG1, OsOTUB1, OsLG3, OsDHHC1, OsY37, qWS8, OsALMT7, GS9, GSN1, OsPS1-F, and OsPK2;(ii) barley genes: vrs4 and COM1 and (iii) maize genes: FASCIATED EAR2, ramosa2, ZmFrk1, bs1, KNR6, and BIF1* (Table 3, S12). Using comparative genomics, orthologs of these genes can be characterized in wheat and their functional markers can be developed and validated. For instance, in a study conducted in 2018, a meta-analysis of QTLs associated with grain weight in tetraploid wheat resulted in the identification of one important locus, mQTL-GW-6A on chromosome 6A. Further analysis identified and characterized a wheat homolog of the rice gene, *OsGRF4* within this MQTL region (Avni et al. 2018). This suggests that integrating an MQTL study with a well-annotated genome can rapidly lead to the detection of CGs underlying traits of interest.

Conclusion

The present study is an effort towards a better understanding of the genetic architecture of grain yield and its component traits in wheat through the identification of MQTLs, orthoMQTLs, and CGs. The study involved an integration of the available information about QTLs that were identified in earlier studies along with genomic and transcriptomic resources of the wheat. As many as 141 MQTLs, each associated with a narrow CI, and 1,202 putative CGs were identified. Thirteen of these 141 MQTLs regions are described as breeder's QTL; we recommend these breeders' QTLs for use in MAS for grain yield improvement in wheat.

The ortho-MQTL analysis demonstrated that MQTLs of yield-related traits appear to be transferable to other cereal crops that may assist breeding programmes in other cereals. Based on a comparative genomic approach, several wheat homologs of rice, barley, and maize yield-related genes were also detected in the MQTL regions. As many as 162 of 1,202 putative CGs are also recommended for future basic studies including cloning and functional characterization. However, after any in-silico analysis of this type, the in-vivo confirmation and/or validation of any of these loci, specifically the CGs identified, is needed, which can be accomplished via further approaches, such as gene cloning, reverse genetic approaches i.e. gene silencing and other approaches including transcriptomics and proteomics etc. The information on the molecular markers linked with the MQTLs and CGs occupying the MQTL region may also prove useful in breeding for grain yield improvement in wheat.

Declarations

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Authors' contribution

D.K.S., P.S. and P.K.G. conceived and designed the project; P.K.G. and P.S. supervised the study; D.K.S. and N.P. conducted the analysis; D.K.S. wrote the paper and P.K.G. and P.S. corrected the final draft. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that they have no competing interests.

Availability of data and material

Data generated or analysed during this study are included in this published article (and its **Supplementary material 1**). Datasets are also available from the corresponding author on reasonable request.

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Figures

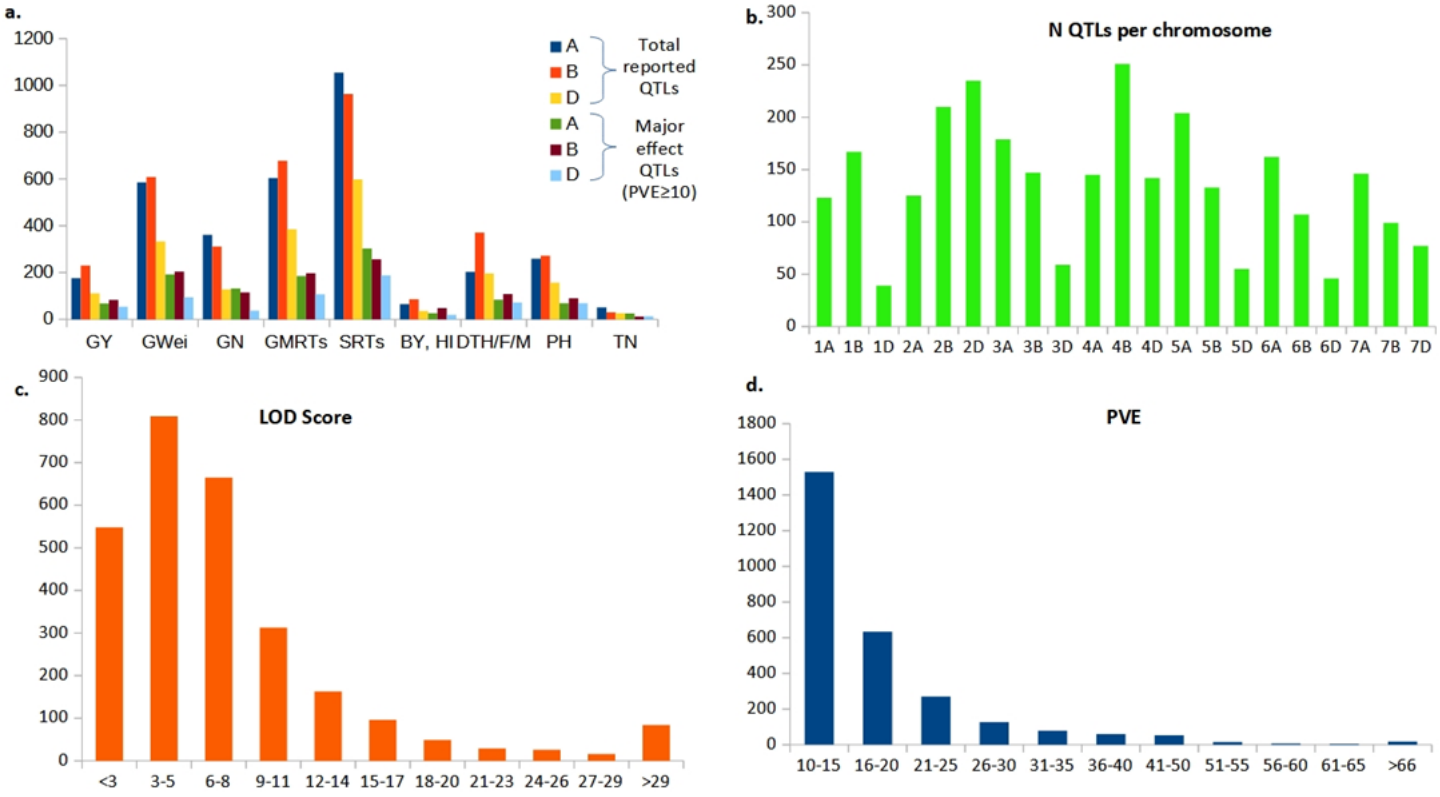

Figure 1

Salient features of the QTLs in terms of frequency distributions using four different criteria: (i) total QTLs and major QTLs on three different sub-genomes (A, B and D); (b) major QTLs on 21 individual chromosomes, (c) major QTLs with different LOD scores, (d) QTLs with different values for phenotypic variation explained (PVE).

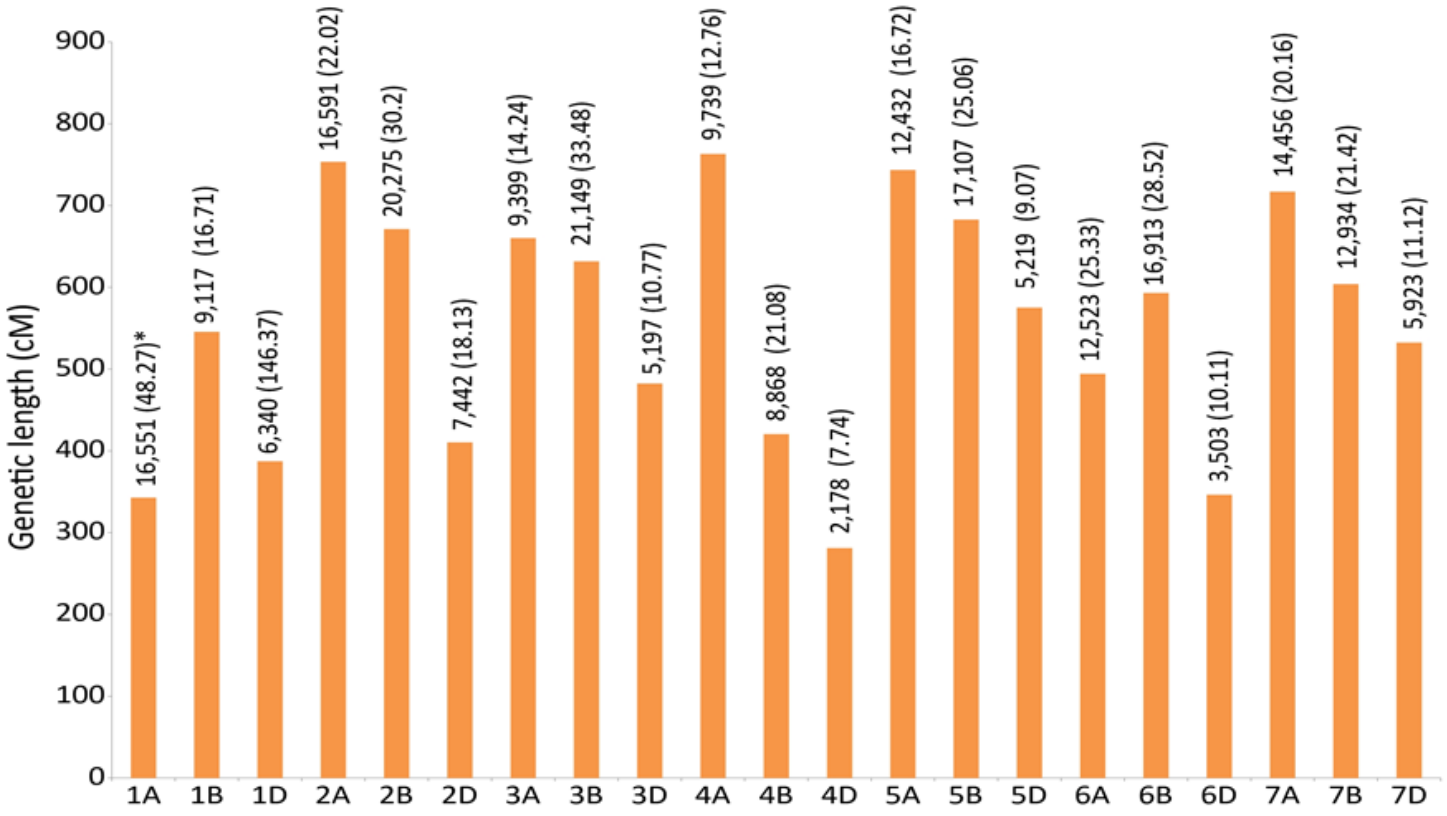

Figure 2

Salient features of the consensus map. *= Number of markers used and marker density per cM (in parenthesis).

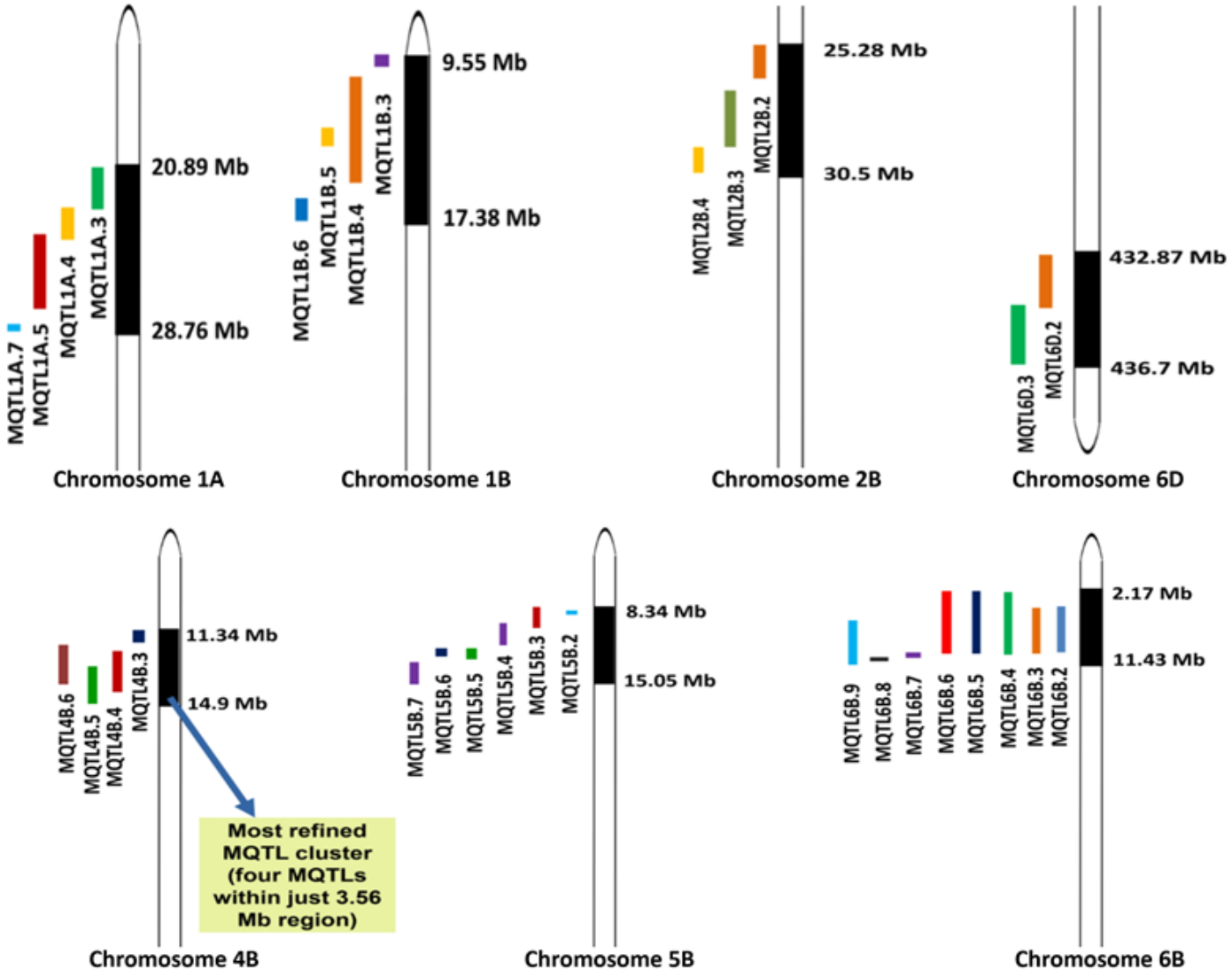

Figure 3

Diagrammatic representation of MQTL clusters detected on chromosomes 1A, 1B, 2B, 4B, 5B, 6B and 6D; Only desired parts of the chromosomes are shown for better visualization; different colours corresponds to different MQTLs on each chromosome.

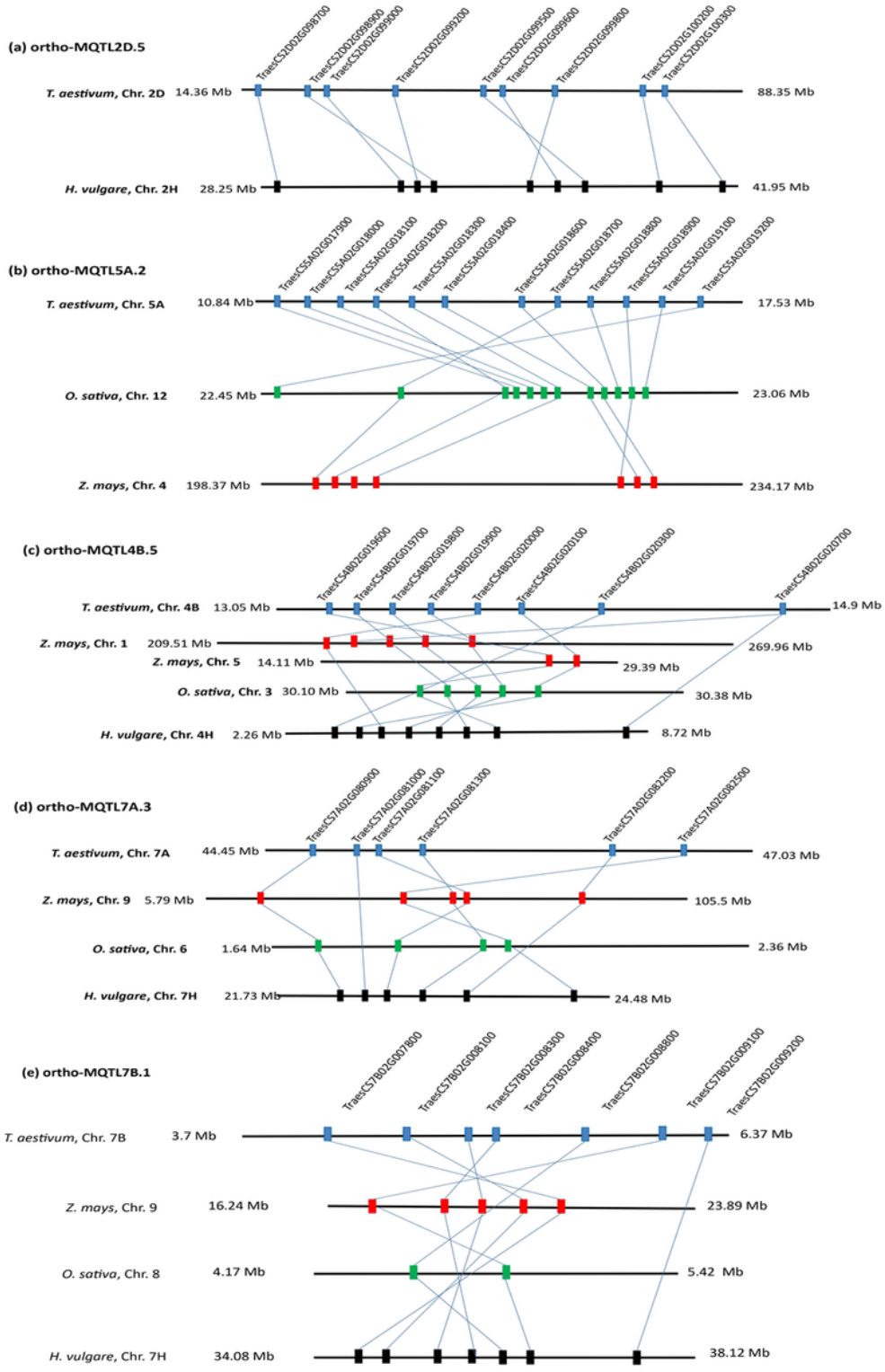

Figure 4

Syntenic regions of five ortho-MQTLs among the wheat, maize, rice, and barley. The chromosome number, genomic position, and common genes among the wheat, maize, rice, and barley are indicated. More details are presented in Table 2.

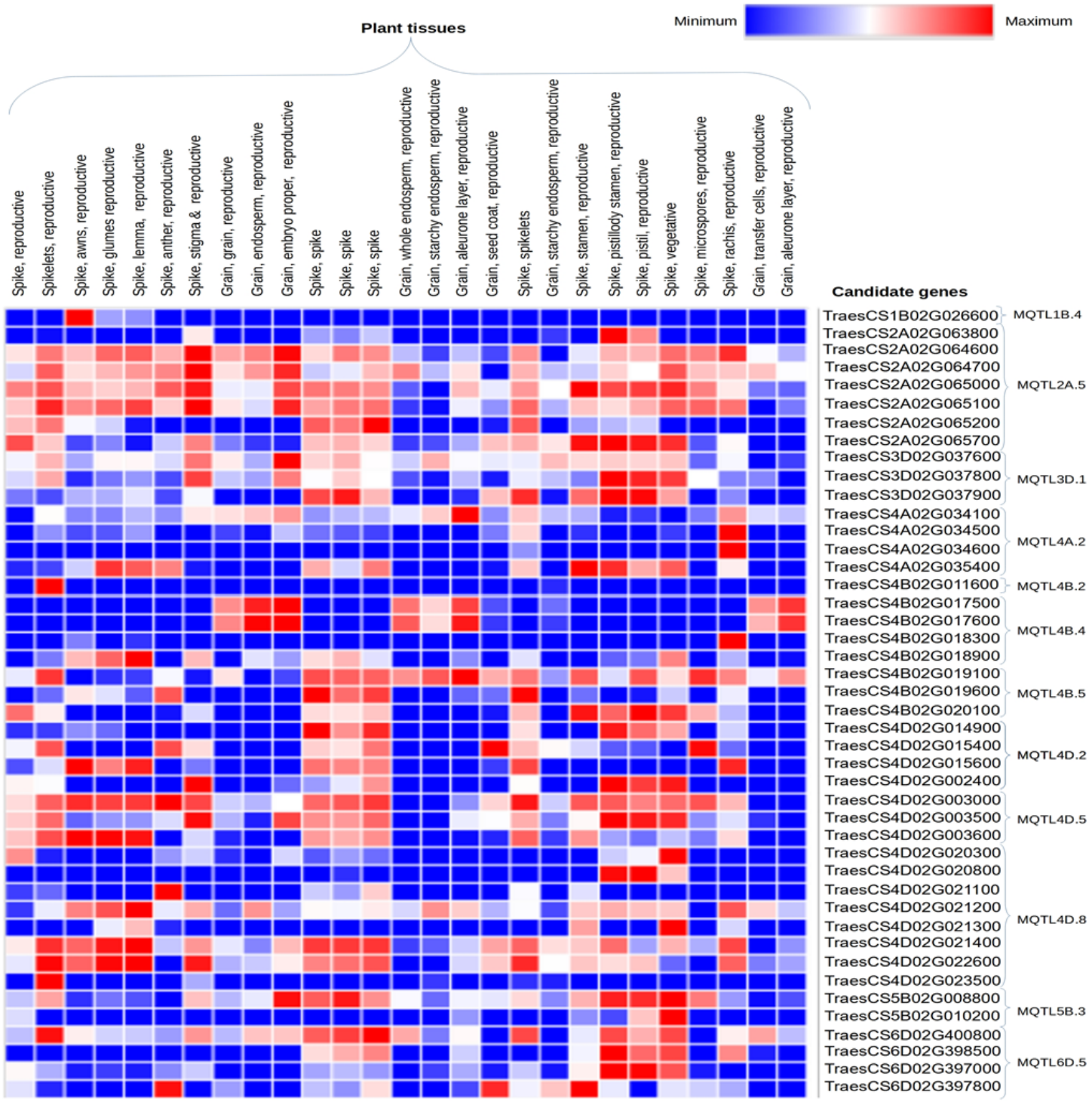

Figure 5

Heatmap showing differential expression of CGs underlying the Breeder's QTLs.

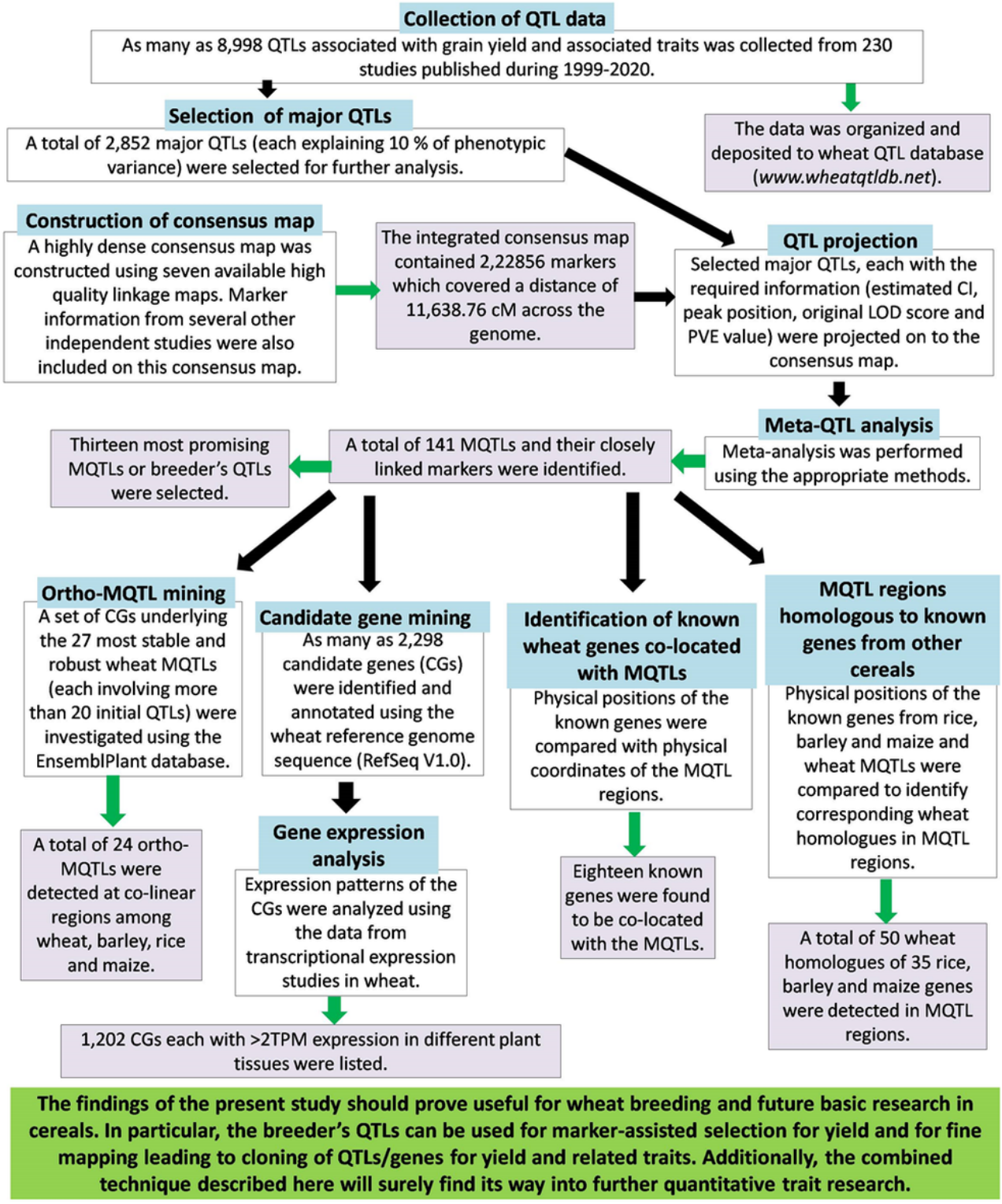

Figure 6

Flow diagram of QTL meta-analysis applied in this study, which further incorporated genomic and transcriptomic publicly available data.

Supplementary Files

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- Supplementarymaterial1.xls